**Laetiporus sulphureus** Affects Migration and Superoxide Anion Radical Level in Hela Cervical Cancer Cells †

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Abstract: Cervical cancer is the fourth most common female malignancy worldwide. Treatment of cancer cells with metastatic potential is an important issue in cancer therapy. In this study, we investigated edible and medicinal mushroom *Laetiporus sulphureus* (Bull.) Murrill with known biological properties for human health. Two selected concentrations (10 and 50 µg/mL) of ethanolic extract of *L. sulphureus* were used to determine the level of superoxide anion radical (NBT test) and migratory potential (Wound healing test) on cervical cancer cell line (HeLa). Effects were measured after 24 and 72 h. The extract induced an acute prooxidative effect on HeLa cells, with a significant reduction in migratory potential of these cells in both tested concentrations. A higher concentration (50 µg/mL) has a slightly stronger antimigratory activity. *Laetiporus sulphureus* is a very important source of biologically active substances and should be reconsidered for development of promising anticancer therapeutics.

Keywords: cervical cancer; wound healing; fungotherapy; NBT assay

1. Introduction

Globally, cervical cancer is the fourth most common cancer that affects women of reproductive and post reproductive age [1–3]. Despite effective prevention and screening, many cervical cancers are detected in the late stages of the disease when metastatic potential of this cell is vitrificated [4].

The mushrooms are remarkable in many respects and are valued as a significant source of many biologically active substances with potential medicinal applications in the treatment of different diseases including cancer [5]. An edible mushroom *Laetiporus sulphureus*, is widely distributed worldwide, except Antarctica. The fruiting body of *L. sulphureus* consists of fleshy, semicircular cap with a striking sulfuric-yellow color [6]. Recently, this commercial mushroom was found to produce a variety of bioactive compounds, with proven medicinal effects. Many secondary metabolites, such as phenolic compounds, polyketides, terpenes, and steroids that have been extracted from the fruiting bodies and mycelium of *L. sulphureus*, have potential anticancer, antimicrobial, anti-inflammatory, immunosuppressor, and antibiotic effects [7,8]. Generally, mushrooms have been mainly evaluated from the perspective of cytotoxicity, and data regarding their effect on the migration of cancer cells are scarce.
In this study we extracted bioactive ingredients of *L. sulphureus* with ethanol solvent and resulting extract was tested on the level of superoxide anion radical and migratory potential of cervical cancer cell line (HeLa).

2. Materials and Methods

*L. sulphureus* was collected in Šumadija area, Serbia, during autumn 2019. Identification and classification of the mushroom were performed by standard keys by mycological society “Šumadija” (Kragujevac, Serbia). Ethanol extract of *L. sulphureus* (LSE) was prepared as described previously by Seklic et al. [9]. For nitroblue tetrazolium test (NBT) 10,000 cells were seeded in each well of 96 well plate, and after 24 h of treatment, cells were treated with two selected concentrations (10 and 50 µg/mL). The level of superoxide anion radical (O$_2^-$) was measured after 24 h and 72 h, on ELISA reader (Elisa Microplate Reader RT-6100, Rayto, China) at 560 nm wavelength.

Wound healing assay was performed when cells reached 90–100% confluency. After treatment with LSE in two selected concentrations (10 and 50 µg/mL) the effects were tracked for 24 h. Micrographs were taken with a microscope (Genetic Digital-Microscope, BNB-300M, China), and the differences in wound closure were measured with ImageJ program.

3. Results

3.1. Effects on Level Superoxide Anion Radicals

We used NBT assay for quantification of superoxide anion radicals’ level in control (untreated) and treated HeLa cells after 24 h and 72 h of incubation. The *L. sulphureus* ethanol extract activated acute prooxidative effects and elevated level of superoxide anion radical in both selected concentrations after 24 h. Furthermore, 72 h after treatment, the level of O$_2^-$ was close to control values (Figure 1).

![Figure 1](image)

**Figure 1.** Effects of LSE treatment on superoxide anion radicals in HeLa cells. Results are presented as means ± standard error.

3.2. Antimigratory Activity

In this study, effects of the extract were evaluated for its antimigratory activity in two selected noncytotoxic concentrations (10 and 50 µg/mL) to avoid the possible interfering cytotoxic effect. In our investigation, LSE notably inhibited the migration of HeLa cells compared to the control value. Concentration 50 µg/mL of this extract exerted a slightly stronger antimigratory effect (Figure 2). The migration of HeLa cells was remarkably inhibited using the tested ethanol extract of *L. sulphureus*.
4. Discussion

The search for natural antioxidants, especially those of mushroom origin, has increased greatly in recent years. Mushrooms usually contain a wide variety of free radical scavenging molecules. Antioxidant activity of extract obtained from mushroom *L. sulphureus* was most probably conditioned by different phenolic compounds of this mushroom species [10]. It was established that ethanol extracts of mushrooms, including *L. sulphureus*, have exerted good antioxidant effects in various investigations [8,10]. It is known that in cells with disturbed redox status, such as cancer cells, polyphenolic compounds can have a significant prooxidative effect [11]. LSE induced an increase in superoxide anion radicals in HeLa cells, especially after 24 h, while after 72 h, the level of reactive species was close to the control level.

The metastasis of cancer is a complex, multistep process that involves degradation of the surrounding extracellular matrix, migration to and proliferation of the cells at a secondary site [12]. Recent studies indicate that the reduction of collective migration of cancer cells is important for the treatment of various types of cancer. Nevertheless, there are few studies on the effect of extracts on collective cell migration [13,14]. However, the ethanol extracts of *L. sulphureus* proved important anticancer effects [15]. Our studies indicate the O$_2^{−}$ production and suppression of cell migration caused by LSE in HeLa cells. According to our preliminary results, inhibition of HeLa cell migration at 24 h is probably a consequence of the acute prooxidative effect of this treatment. Earlier research confirmed that extracts of different types of mushrooms caused oxidative stress, and also a pronounced antimigratory effect on cancer cell lines [9], which is in concordance with our results. Our future research will be focused at explaining the mechanism of action of this extract.

5. Conclusions

Tested *L. sulphureus* extract showed acute prooxidative effect and inhibitory potential on migration of HeLa cells. *L. sulphureus* is a very important source of biologically active substances and should be reconsidered for development of promising anticancer therapeutics. Newly, more detailed researches are necessary.

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**Figure 2.** Effects of LSE on migration of HeLa cell line. Analysis of wound space is shown as relative level of changes of wound space width. Results are presented as means ± standard error.
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