


Proceeding Paper

Laetiporus sulphureus Affects Migration and Superoxide Anion Radical Levels in HeLa Cervical Cancer Cells [†]

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

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



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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 vitrified [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]. The edible mushroom *Laetiporus sulphureus* is widely distributed in the world, except in Antarctica. The fruiting body of *L. sulphureus* consists of a fleshy, semicircular cap with a striking sulfuric-yellow color [6]. Recently, this commercial mushroom has been found to produce a variety of bioactive compounds with proven medicinal effects. Many secondary metabolites that have been extracted from the fruiting bodies and mycelium of *L. sulphureus*, such as phenolic compounds, polyketides, terpenes, and steroids, showed to possess potent anticancer, antimicrobial, anti-inflammatory, immunosuppressive, 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 an ethanol solvent and the resulting extract was tested on the level of superoxide anion radical and migratory potential of a cervical cancer cell line (HeLa).

2. Materials and Methods

L. sulphureus was collected in Šumadija area, Serbia, in autumn 2019. The identification and classification of the mushroom were performed using standard keys by mycological society “Šumadija” (Kragujevac, Serbia). The ethanol extract of *L. sulphureus* (LSE) was prepared as described previously by Šeklić et al. [9]. For nitroblue tetrazolium test (NBT), 10,000 cells were seeded in each well of a 96-well plate, and after 24 h of treatment, the cells were treated with two selected concentrations (10 and 50 µg/mL). The level of superoxide anion radical ($O_2^{\cdot-}$) was measured after 24 h and 72 h on an ELISA reader (Elisa Microplate Reader RT-6100, Rayto, Guangdong, China) at 560 nm wavelength.

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

3. Results

3.1. Effects on Superoxide Anion Radicals Level

We used the NBT assay for the quantification of superoxide anion radical levels in the control (untreated) and treated HeLa cells after 24 h and 72 h of incubation. The *L. sulphureus* ethanol extract activated the acute pro-oxidative effects elevating the level of superoxide anion radical in both selected concentrations after 24 h. Furthermore, 72 h after treatment, the level of $O_2^{\cdot-}$ was close to the control values (Figure 1).

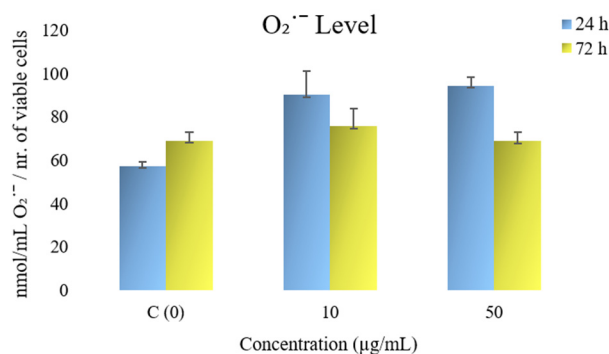


Figure 1. Effects of the LSE treatment on superoxide anion radicals in HeLa cells. Results are presented as means \pm standard error.

3.2. Antimigratory Activity

In this study, the effects of the extract were evaluated for its antimigratory activity in two selected noncytotoxic concentrations (10 and 50 µg/mL) to avoid the possible interference of a cytotoxic effect. In our investigation, LSE notably inhibited the migration of HeLa cells compared to the control values. The 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*.

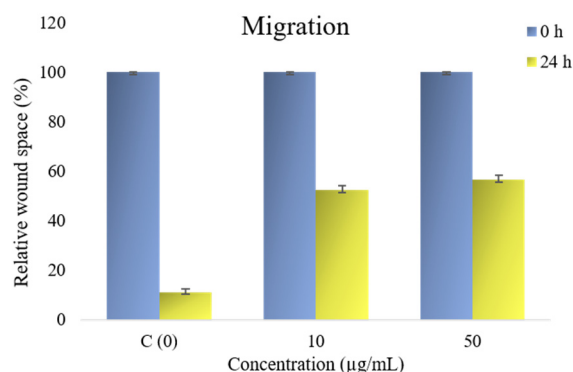


Figure 2. Effects of LSE on the migration of the HeLa cell line. Analysis of wound space is shown as relative level of changes in wound space width. Results are presented as means \pm standard error.

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. The antioxidant activity of the extract obtained from the mushroom *L. sulphureus* was most probably conditioned by different phenolic compounds of this mushroom species [10]. It has been established in various investigations that ethanol extracts of mushrooms, including *L. sulphureus*, exert good antioxidant effects [8,10]. It is known that, in cells with a disturbed redox status, such as cancer cells, polyphenolic compounds can have a significant pro-oxidative 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 the degradation of the surrounding extracellular matrix, migration and proliferation of the cells at a secondary site [12]. Recent studies have indicated that the reduction in the collective migration of cancer cells is important for the treatment of various types of cancer. Nevertheless, there are few studies regarding the effect of extracts on collective cell migration [13,14]. However, the ethanol extracts of *L. sulphureus* proved important anticancer effects [15]. Our studies have indicated the $O_2^{\cdot -}$ production and suppression of cell migration caused by LSE in HeLa cells. According to our preliminary results, the inhibition of HeLa cell migration at 24 h is probably a consequence of the acute pro-oxidative effect of this treatment. Earlier research has 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 on explaining the mechanism of action of this extract.

5. Conclusions

The tested *L. sulphureus* extract showed an acute pro-oxidative effect and inhibitory potential on the migration of HeLa cells. *L. sulphureus* is a very important source of biologically active substances and should be reconsidered for the development of promising anticancer therapeutics. Therefore, more detailed research is necessary.

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