Foods 2021

MDPI

Proceeding Paper

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

Milena M. Jovanović [1,*], Katarina Virijević [2], Jelena Grujić [2], Marko Živanović [2] and Dragana S. Šeklić [2]

[1] Department for Biology and Ecology, Faculty of Science, Radoja Domanovića 12, University of Kragujevac, Serbia

[2] Institute for Information Technologies Kragujevac, Department of Natural Sciences, Jovana Cvijića bb, University of Kragujevac, Serbia; msc.katarina.virijevic@gmail.com (K.V.); jelena.grujic2@aiesec.net (J.G.); zivanovicmkg@gmail.com (M.Ž.); ddjacic@yahoo.com (D.S.Š.)

* Correspondence: milena.jovanovic@pmf.kg.ac.rs

† Presented at the 2nd International Electronic Conference on Foods, 15–30 October 2020; Available online: https://foods2021.sciforum.net/.

Abstract: Colorectal and cervical cancer are major health problem worlwide, and adjuvant therapy which includes fungi is considered valuable in cancer treatment. Herein, we evaluated effects of edible mushroom species *Laetiporus sulphureus* on 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 different manner. However, presented effects of this mushroom should not be neglected in future studies, esspecially regarding more detailed studies and drug development.

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

Citation: Jovanović, M.M; Virijević, K.; Grujić, J.; Živanović, M.; Šeklić, S.D. Extract of Edible Mushroom *Laetiporus sulphureus* Affects Redox Status and Motility of Colorectal and Cervical Cancer Cell Lines. *Proceedings* **2021**, *68*, x. https://doi.org/10.3390/xxxxx

Published: 15 October 2021

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Copyright: © 2021 by the authors. Submitted for possible open access publication under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).

1. Introduction

Throughout the years, colorectal cancer (CRC) has become the third most common diagnosed cancer globally and, 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 biactive substances with already proven anticancer activity are proposed to reduce in significant manner 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 cure the patients with cancer, also helping in improvement of quality and prolongation of their lives.

Almost all cancers are proved to possess elevated level of reactive oxygen species (ROS) which are actually second messengers in cellular signal pathways, and are responsible for promotion of various aspects of cancer genesis and function. In accordance with the foregoing, antioxidant proteins (i.e., non-enzymatic molecule–glutathione), with important role in detoxification from ROS, are confirmed to be also at higher rate in cancer [4]. Reactive oxygen species, such as superoxide anion radicals or nitrite oxides are directly causing 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 aproaches in drug discovery and cancer treatment [4].

Mushrooms have been traditionally used worldwide for treating various ailments, and *Laetiporus sulphureus*, as an edible mushroom, posess various medicinal properties, such as antioxidative and anticancer [5]. Present study was conducted to evaluate effects of *L. sulphureus* ethyl-acetate extract (**LSEA**) on cancer cells viability and 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]. Determination of superoxide anion radicals (O_2^-), nitrites (NO_2^-) and reduced glutathione (GSH) was done using NBT, Griess and GSH methods [6]. For 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 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 percents (mean ± SE). * $p >$ 0.005 was considered as 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).

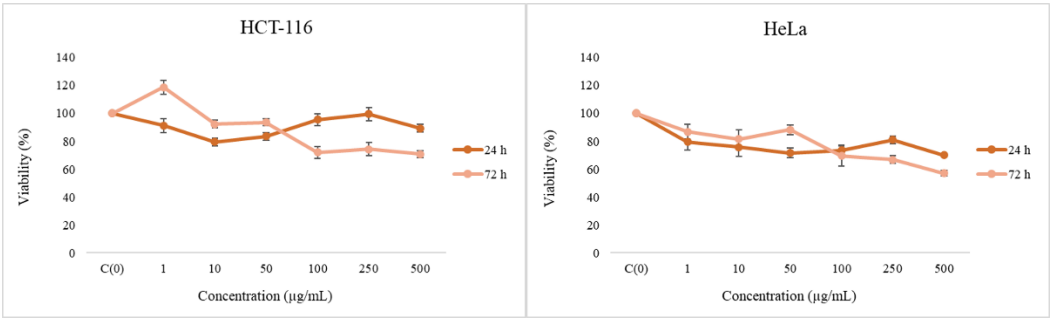

Figure 1. Effects of **LSEA** on HCT-116 and HeLa cell lines viability, 24 and 72 h after treatment.

However, their redox status parameters were affected, whereat **LSEA** elevated both ROS and RNS in HCT-116 cells in acute manner, while after longer time of exposure 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). Response of HCT-116 cells to treatment in form 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** in means of triggered overproduction of $O_2^{\cdot-}$, while level of nitrites remained almost unchanged. Meanwhile, GSH concentration was slightly heightened by applied treatment in these cells (Figure 2).

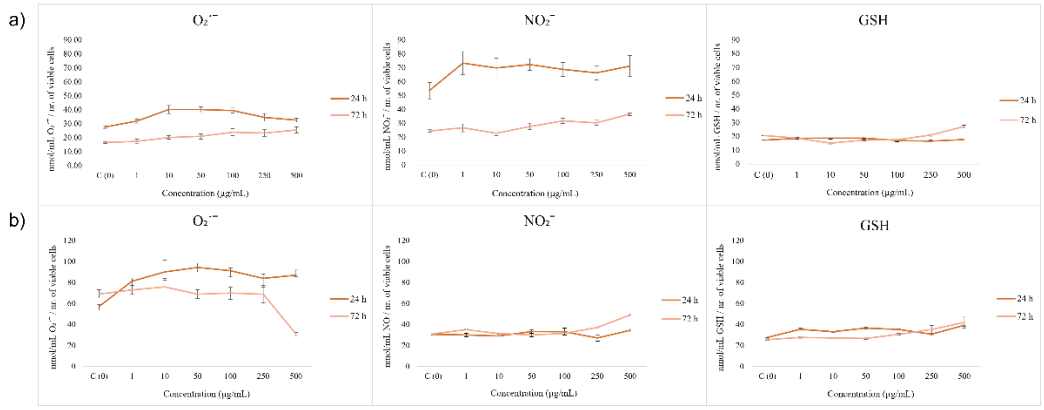

Figure 2. Effects of **LSEA** on redox status parameters in HCT-116 (**a**) and HeLa (**b**) cells, 24 and 72 h after treatment.

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

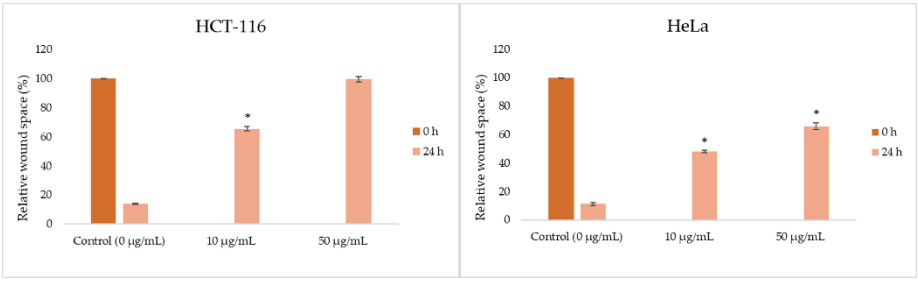

Figure 3. Suppression of HCT-116 and HeLa cells motility by **LSEA** extract measured after 0 and 24 h of treatment.

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

Literature data reported different results when it comes to relation between changes in ROS level and the migratory potential. Namely, increased ROS level can both stimulate and cause supression of mobility of cancer cells [9,10]. Moreover, study conducted earlier showed that an increase in ROS and RNS level 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, esspecially in prevention of cancer.

Author Contributions: Conceptualization, D.S.Š.; methodology, M.M.J. and D.S.Š.; software, K.V.; validation, D.S.Š.; formal analysis, J.G.; investigation, M.M.J.; resources, M.Ž.; data curation, D.S.Š.; writing—original draft preparation, M.M.J. and D.S.Š.; writing—review and editing, D.S.Š. and M.Ž.; visualization, K.V and J.G.; supervision, D.S.Š. All authors have read and agreed to the published version of the manuscript.

Institutional Review Board Statement:

Informed Consent Statement:

Data Availability Statement:

Acknowledgments: This research was funded by MINISTRY OF EDUCATION, SCIENCE AND TECHNOLOGICAL DEVELOPMENT OF THE REPUBLIC OF SERBIA, grant number 451-03-9/2021-14/200378 and 451-03-9/2021-14/200122.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

1. Florescu-Ţenea, R.M.; Kamal, A.M.; Mitruţ, P.; Mitruţ, R.; Ilie, D.S.; Nicolaescu, A.C.; Mogoantă, L. Colorectal cancer: An update on treatment options and future perspectives. *Curr. Health Sci. J.* **2019**, *45*, 134–141. https://doi.org/10.12865/CHSJ.45.02.02.
2. Ahmed, M. Colon cancer: A clinician's perspective in 2019. *Gastroenterol. Res.* **2020**, *13*, 1–10. https://doi.org/10.14740/gr1239.
3. Zhang, S.; Xu, H.; Zhang, L.; Qiao, Y. Cervical cancer: Epidemiology, risk factors and screening. *Chin. J. Cancer Res. = Chung-Kuo Yen Cheng Yen Chiu* **2020**, *32*, 720–728. https://doi.org/10.21147/j.issn.1000-9604.2020.06.05.
4. Liou, G.Y.; Storz, P. Reactive oxygen species in cancer. *Free Radic. Res.* **2010**, *44*, 479–496. https://doi.org/10.3109/10715761003667554.
5. Younis, A.M.; Yosric, M.; Stewarte, J.K. In vitro evaluation of pleiotropic properties of wild mushroom *Laetiporus sulphureus*. *Ann. Agric. Sci.* **2019**, *64*, 79–87.
6. Šeklić, D.S.; Stanković, M.S.; Milutinović, M.G.; Topuzović, M.D.; Štajn, A.Š.; Marković, S.D. Cytotoxic, antimigratory, pro-and antioxidative activities of extracts from medicinal mushrooms on colon cancer cell lines. *Arch. Biol. Sci.* **2016**, *68*, 93–105.
7. Kosanić, M.; Šeklić, D.S.; Jovanović, M.M.; Petrović, N.; Marković, S. Original article—Hygrophorus eburneus, edible mushroom, a promising natural bioactive agent. *EXCLI J.* **2020**, *19*, 442–457.
8. Hassen, S.; Ali, A.A.; Kilaparty, S.P.; Al-Anbaky, Q.Q.; Majeed, W.; Boman, B.M.; Fields, J.Z.; Ali, N. Interdependence of DNA mismatch repair proteins MLH1 and MSH2 in apoptosis in human colorectal carcinoma cell lines. *Mol. Cell. Biochem.* **2016**, *412*, 297–305. https://doi.org/10.1007/s11010-015-2636-3.
9. Fini, M.A.; Orchard-Webb, D.; Kosmider, B.; Amon, J.D.; Kelland, R.; Shibao, G.; Wright, R.M. Migratory activity of human breast cancer cells is modulated by differential expression of xanthine oxidoreductase. *J. Cell Biochem.* **2008**, *105*, 1008–1026. https://doi.org/10.1002/jcb.21901.
10. Nishikawa, M. Reactive oxygen species in tumor metastasis. *Cancer Lett.* **2008**, *266*, 53–59. https://doi.org/10.1016/j.canlet.2008.02.031.