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Biologically active compounds from edible mushroom: Effect on HeLa cells

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ABSTRACT

Background*:* Mushrooms are unique organisms rich in proteins, carbohydrates, vitamins, and minerals, which allows them to be used not only as a source of nutrition but also in cosmetics and medicine. The fungus *P. ostreatus* possesses two strong enzymatic complexes – lignin-decaying and cellulose-decaying.

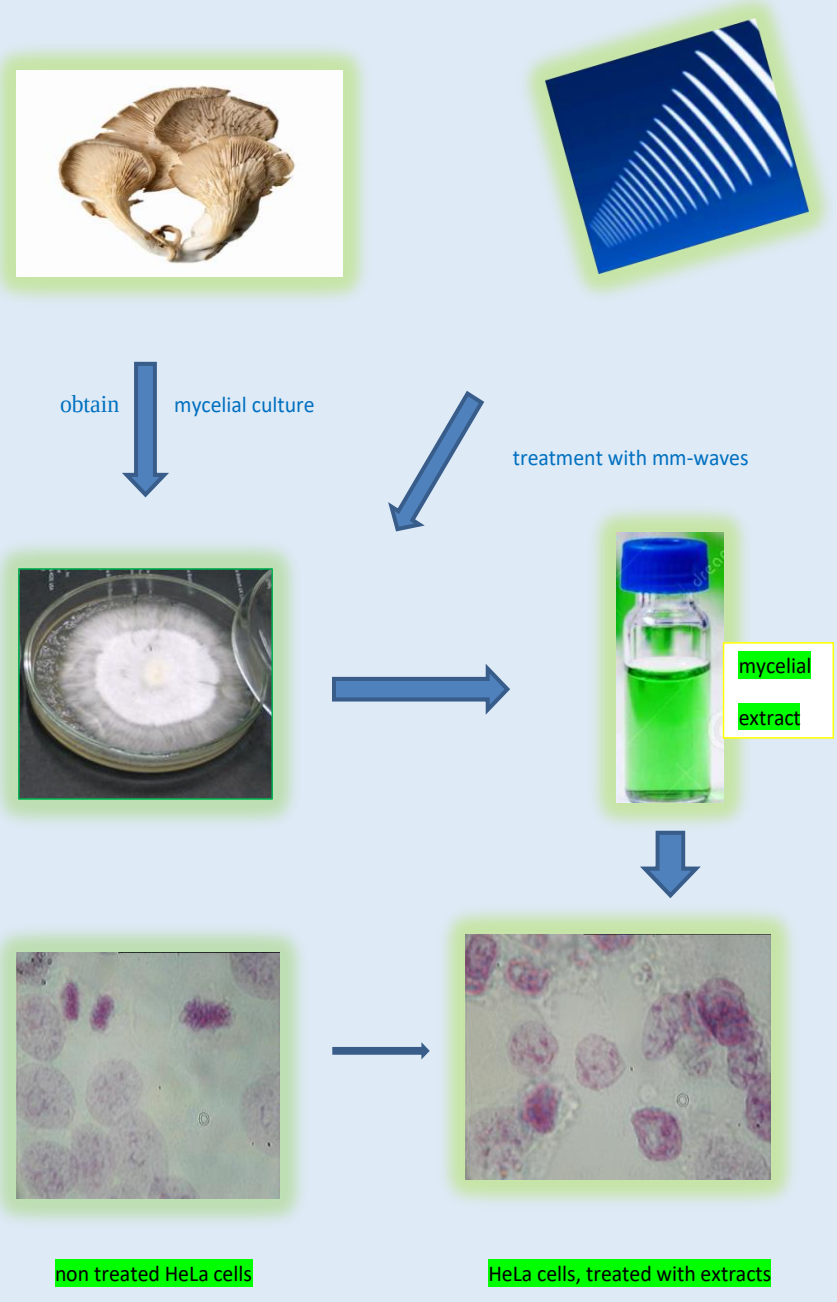
Objective: The research aimed to study the antitumor activity of treated mm-wave mycelial extracts of mushrooms on the HeLa cell line. Research on edible mushrooms against tumors is very actual and important for the field of functional food science and bioactive compounds field.

Methods: The effect of the fungal culture extract on the cyto-photometric and morphometric indices of the HeLa cell line culture was studied according to the nature of proliferation and transcription of malignant tissues. Using high-resolution liquid chromatography, the amino acid composition of acid-soluble proteins in mycelial extracts of a mushroom culture treated with mm waves was studied.

Results: Extracts of *P. ostreatus* mycelial culture significantly suppressed the growth of proliferative activity of human tumor cultures and completely suppressed the mitotic activity of continuous HeLa cell cultures to 72 hours. Our study revealed a significant increase in glutamic and aspartic acids in mycelial culture extracts treated by mm-waves.

Conclusion: Based on HPLC analysis, we suggest polyfunctional peroxidase as the candidate for the role of biologically active compound in the composition of aqueous extracts of the mycelial culture. So, the polyfunctional peroxidase plays an active role in the therapeutic properties of the oyster mushroom.

Keywords: anticancer activity, ant-inflammation activity, mm-waves, mushroom`s, mycelial extracts, HPLC of acid-soluble proteins, HeLa cell line.



Graphical Abstract: Biological active compounds from edible mushroom: Effect on Hela cells

INTRODUCTION

Natural products used in the pharmaceutical industry have had great success because they have no adverse side effects. At the same time, canned foods, large amounts of meat and smoked sausages, alcoholic and carbonated drinks, and high-fat foods increase the likelihood of developing not only gastrointestinal tumors but also some other types of cancer [1]. The basis of all functional foods are bioactive compounds that enter the body with plant and animal foods, which have a beneficial and healing effect on the human body [2]. Basidial fungi contain many biologically active compounds, such as minerals, folates, lectins, polysaccharides, fats, proteins, glycosides, alkaloids, essential oils, carotenoids, terpenoids, tocopherols, flavonoids, phenols, as well as enzymes and ascorbic acid are contained in macromycetes. Medicinal mushrooms also contain organic acids, which are responsible for more than 100 medicinal functions of mushroom extracts. Besides dietary habits, the list of risk factors includes family medical history, tobacco use, infections as Helicobacter pylori, and Epstein-Barr virus, human papillomavirus (HPV) responsible for many types of cancers [3]. Generally, most mushrooms contain high amounts of vitamin D, vitamin B, ω-3 PUFA, plant fiber, phytoestrogen, and folate, which can prevent breast cancer [4]. There is a wide range of useful wood-decaying mushrooms, which possess two base systems of ferments, cellulose-destroying and lignin-destroying ferments system. Glucans and specific proteins are responsible for most of the mushrooms' immunomodulatory and antitumor therapeutic properties. Fungal immunomodulatory proteins (FIPs), lectins, as well as ribosome-inactivating proteins (RIPs), ribonucleases and laccases, and other proteins with bioactive effects, are contained in basidiomycetes [5]. The vast majority of mushrooms are 85–95% water, contain 35–70% carbohydrates and 17–34.7% proteins, including essential ones, as well as a small amount of fats and mineral compounds (such as selenium, potassium, and phosphorus) (6–10.9%), and nucleic acids (3–8%); rich in vitamins B1 (thiamine) 1.4–2.2 mg (%) and vitamin B7 (biotin), riboflavin (vit. B2) 6.9–8.9 mg (%) and niacin (vitamin B3) 60.6–73.3 mg (%), vitamin C (ascorbic acid) 92–144 mg (%), pantothenic acid (vit. B5) 21.1–33.3 mg (%) and vitamin B9 (folic acid) 1.2–1.4 mg/100 g in dry weight basis [6]. Given the rich protein and vitamin content of P. ostreatus, it can be expected that they are not only healthy and nutritious but can also prevent or slow down many types of inflammatory and precancerous conditions, suppress the activity of viruses, including the human papillomavirus (HPV). Medicinal mushrooms can increase the effectiveness of vaccination against many diseases.

Some current reviews noted about the anti-inflammatory and anticancer activity of some Basidiomycetes [7], the efficiency of mycochemicals against cancer stem cells, the notable influence of different extracts from mushrooms on neuro-inflammation and common on the alleviating of neurological disorders [8, 9]. It has been mentions its connection between mushrooms bioactive compounds with immunity [5] and its favorable effects in diabetes mellitus [10]. Additionally, it has shown its antigenotoxic effects [11], its potential as a prebiotic, and even its potential to reduce adverse effects from chemotherapy and radiotherapy of people [12-13]. Mushrooms are also credited with several medicinal properties, such as antibacterial and antiparasitic activity, anti-inflammatory and hepatoprotective activity, they have antioxidant and detoxifying properties, but the most important of these functions are antiviral and anticancer activity [3, 14].

Proteins and glucans contained in P. ostreatus are capable of exerting antiviral and anti-inflammatory effects, due to the content of antioxidant compounds, they reduce the risk of hypocholesterolemia and have an

antiatherogenic neuroprotective effect, have antitumor and immunomodulatory properties, and can also neutralize free radicals in the human body [1, 6]. Among them, biologically active compounds such as α- and β-glucans, lentinan, lipopolysaccharide resveratrol, concanavalin A, natural statin mevinolin, and many others were found in these mushrooms [5, 15]. Most of the therapeutic and antitumor activity is considered with these compounds of biologically active substances in P. ostreatus. At the same time, HPV infections are detected in tumor tissues in 90% of cases of cervical and anal cancer [16]. We have previously shown high antioxidant and anti-inflammatory activity of extracts of the mycelial culture of this fungus, enhanced by exposure to mm waves, which is promising for treating cancer caused by viral infection [17-19].

The aim of the research to study extracts with a high therapeutic effect, isolated from a mycelial culture of edible mushroom *P. ostreatus*, for their anticancer activity on the continuous HeLa cell line. To obtain the more high-activity extract, we have modulated conditions of mycelial growth by treating it with mm waves of different frequencies. This work aims to evaluate the anticancer activity of extracts isolated from mm-wave-activated mycelial cultures of P. ostreatus and to identify the nature of the protein compounds responsible for this activity.

MATERIALS AND METHODS

The object of the study was basidiomycetes – freshly harvested oyster mushroom – *Pleurotus ostreatus* (P. Kumm.), collected in the deciduous forests of the Ijevan floristic region of Armenia. Freshly harvested fruiting bodies were used to obtain pure mycelial cultures. Oyster mushrooms (*Pleurotus ostreatus*: P. Kumm.) in Armenia grow in all floristic regions from 500 to 2800 m above sea level, abundantly, in groups. Under natural conditions, *P. ostreatus* is found in deciduous and mixed forests on living and dead trunks, stumps, and dead wood of deciduous trees, less often of coniferous species. It is also artificially grown on sawdust, straw, sunflower husks, corn cobs, flax hemp fire, and other waste, causing white rot (Fig.1). The therapeutic properties of mushrooms are dependent on the type of growth substrate and conditions [20-21]

Fig.1. Oyster mushrooms in nature and artificially grown on sawdust.

Obtaining pure cultures of wood-decaying fungi: Pure cultures from oyster mushroom fruiting bodies were obtained by the tissue method from a fragment of the carpophore of the fruiting body, which were placed in the

center of a Petri dish with wort agar. As a nutrient medium for obtaining mycelial cultures, we used agarized beer wort (20, 40, 80 according to Balling), pH 4.5-5, with the addition of 200 units of penicillin or 100 units of another antibiotic per liter of medium, to prevent fungal cultures from accompanying bacterial microflora. Petri dishes with inoculum were placed in a thermostat for cultivation at a temperature of 24-26^{0}C.

Preparation of mycelial extracts: The resulting mycelium was separated from the agar, weighed, and ground in a pre-cooled mortar with glass beads, adding 0.15 M Tris-HCl buffer, pH = 8.0 (Reanal, Hungary). Buffer added at the rate of 0.2 ml of buffer per 100 mg of mycelium. The extract was separated by centrifugation in the cold for 20 min at 18,000 rpm on a high-speed centrifuge type 310b (Mechanika Precyzyjna, Poland). The supernatant liquid was used for subsequent analysis of the quantity of protein, enzymatic activities of the mushroom extracts, and studies of anti-inflammatory and anticancer activity.

Irradiation of mushroom mycelium with mm waves: Mycelial cultures of fungi *P. ostreatus*, on the 7th day of growth, were treated with mm waves lasting 20 and 40 minutes, with a radiation power of 0.64 mV/cm^2. After a single irradiation, the cultures continued to be grown in a thermostat at 25^{0}C for another 3-4 days to obtain sufficient mycelium biomass, from which intracellular fungal extracts were obtained for further study. An extract of a non-irradiated fungal culture was used as a control.

HPLC of protein mixtures from mushroom extracts: Analysis of amino acids from mycelial fungal extracts was studied using high-performance liquid chromatography (HPLC) with electrochemical detection on an isocratic HPLC device "Biochrome" (Moscow, Russian Federation). The chromatographic system included a standard analytical column 150×4.6 mm containing the carrier "Nucleosil 100-5 C 18", an electrochemical detector DE-108 (Russia), a pump for HPLC "Marathon" series II, an injector for introducing a sample "Rheodyne" model 7725J and Multikhrom computer processing system (Ampersand, Russia) [22]. The potential of the working electrode versus the reference electrode was 850 mV. The mobile phase was a mixture of phosphate buffer (pH 5.6): methanol in a volumetric ratio of 25:1, with an elution rate – 1 ml/min. Aspartic acid, glycine, taurine, gamma-aminobutyric acid, and glutamic acid (Sigma, Germany) in 0.1N $HClO_4$ were used as standards, as presented in the Fig. 2.

To analyze mycelial extracts, 50 µL of a diluted "internal standard" solution and 50 µL of orthophthalaldehyde solution were added to 50 µL of extract. All values corresponding to amino acids were automatically subjected to spectral analysis by comparing the spectra of commercial drugs and derivatives using the Multichrome program.

Determination of anti-cancer activity of extracts: Imaging systems can use several nuclear morphometric descriptors that help detect the level of transformation in a cell population. Nuclear morphological changes associated with loss of differentiation correlate with tissue transformations. As shown in research, transformed cells are usually characterized by aneuploidy nuclei, which are manifested by a high degree of variability in size and shape and an increase in the number of enlarged nucleoli. The studies were carried out according to the methodology of Z.A. Karalyan [23]. HeLa tissue cells were cultured in Eagle's medium supplemented with 10% bovine serum. Cells were sub-cultured once at a dose of 105 cells/ml. Each medium contained 2 mM L-glutamine, 1 mM sodium pyruvate, 100 U penicillin/ml, and 0.1 mg/ml streptomycin. Monolayers of intact cells were examined under a microscope connected to a computer 48 and 72 hours

after subculture incubation with and without aqueous mycelial extract (control). Studying the effects of fungal extracts on cancer cell lines in vitro may shed light on their therapeutic properties.

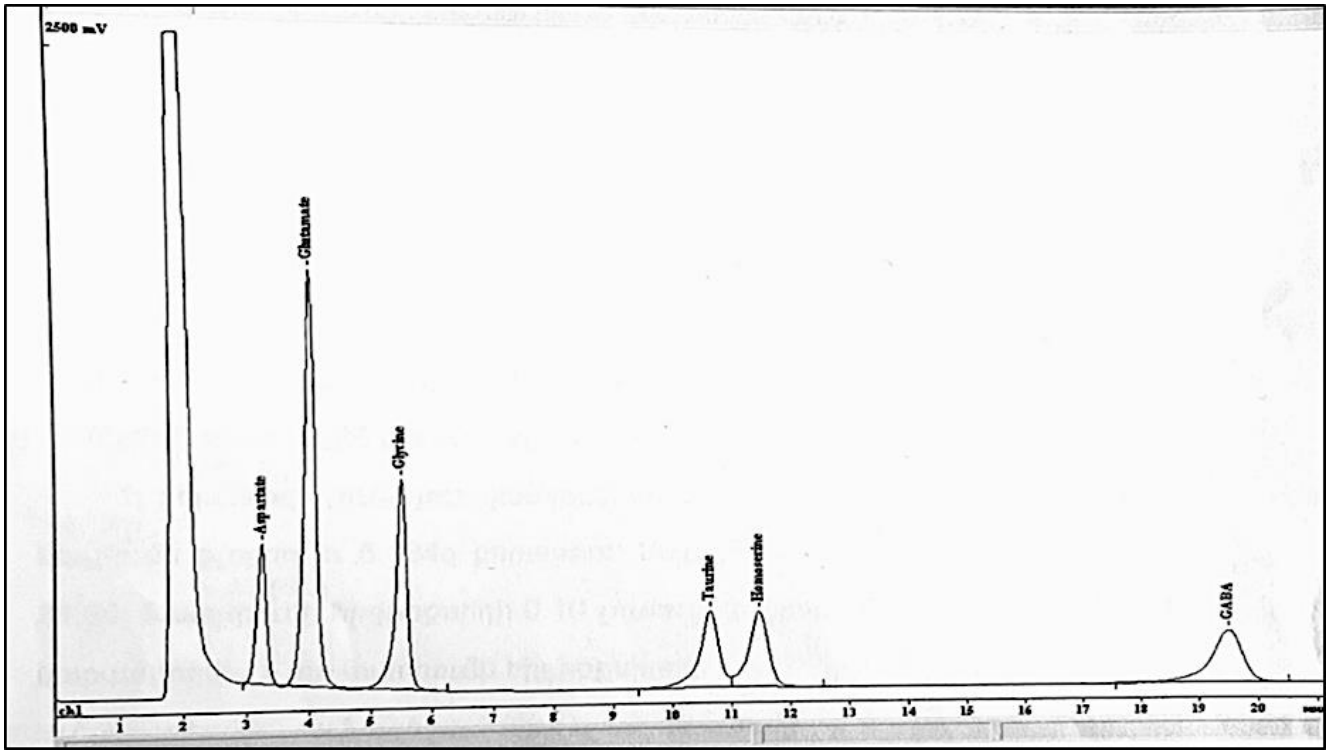


Fig.2 Standards of amino acids in chromatography.

Statistical data processing: The diagrams show arithmetic means and their standard deviations from 4 biological replicates of experiments and 2 analytical replicates for each of them. When processing data using the Student t-test method, confidence intervals were determined for the average impact of EHF EMR at a 5% significance level. Data that did not fall within the confidence interval in Tables 1 and 2 are marked with asterisks. An analysis of the variance of a two-factor complex was also carried out, which confirmed the reliability of the obtained changes both in the frequency and duration of exposure to mm waves and in their combined influence (Fs = 6.24 and Fs = 4.84, respectively). Mathematical processing of research results was carried out using Microsoft Excel functions.

RESULTS AND DISCUSSION: The structure of the secondary cell walls of the wood fungus – P. ostreatus consists of chitin, rich in glucans and lignin (a polymer of phenylpropanoids). It has been established that all phenolic compounds play an important role in processes such as photosynthesis, respiration and growth, and plant resistance to various diseases. The mushrooms are rich in β-glucosidase and various peroxidases, which are necessary to synthesize and break down lignin and polysaccharides and their derivatives. We have previously shown that at certain frequencies of mm-wave, some fungal enzymes are quickly activated [18-19]. Therefore, we have assumed that the extract from the fresh culture of the mushroom *P. ostreatus* will be effective as an anti-inflammatory drug. We have tested extracts from untreated and treated mm-waves mycelial cultures of *P. ostreatus* with modulated enzymatic activity on a rat's acute ear model. In addition, comparative studies of the anti-inflammatory activity of extracts obtained from cultures of other types of wood-decaying fungi - *Lentinula edodes* (shiitake) and

Ganoderma lucidum (reishi), presented earlier in the article [19] were carried out. The experimental results indicate that at a frequency of 50.3 GHz, the maximum anti-inflammatory activity of extracts from mycelial cultures of *P. ostreatus* is achieved. The results of these experiments once again confirm our assumption about the relationship between anti-inflammatory activity and the activity of fungal peroxidases. The results were especially clearly obtained using the example of oyster mushrooms since this particular mushroom has high antioxidant activity [24]. Exposure to mm waves causes an increase in the activity of peroxidases, which allowed us to identify and confirm the relationship between anti-inflammatory and peroxidase activities (but not correlated with betta-glucosidase activity) in the extract of mycelial cultures of oyster mushroom, which is to some extent valid for extracts of cultures of three studied species of wood-decaying mushrooms [18-19].

The data we obtained indicate that the extract of oyster mushrooms has pronounced anti-inflammatory activity, which, according to our data, is due to the presence of lignin-lytic enzyme systems in the extract [25]. Many reviews discuss the role of one or another compound from various fungi in anti-cancer activity [3-5]. As we expected, it was the high antioxidant activity of oyster mushrooms that correlated with more effective anti-inflammatory activity with reishi and shiitake, and it is this activity that is capable of exerting the anticarcinogenic effect of the extract on tumor tissue cells.

According to the obtained data, an extract of the oyster mushroom culture, treated with mm waves at a frequency of 50.3 GHz, suppresses the inflammatory process by 87.5% within 1-2 hours, and treatment with this frequency corresponds to the highest antioxidant - peroxidase activity [17]. According to Virchow's assumption, drugs with high anti-inflammatory activity should also have an anti-carcinogenic effect [26]. We also studied the antiproliferative activity of the mycelial extract of P. ostreatus in vitro. Stimulation of the immune defense system by biologically active polymers contained in medicinal mushrooms has a significant effect on the maturation, differentiation, and proliferation of many types of human immune cells; fungal polymers have immunotherapeutic properties, helping to suppress growth and destroy tumor cells [27]. Table 1 presents the data on the inhibition of HeLa cell line proliferative activity under treatment by an extract from mycelial cultures. As evidenced by our data, in the control line, untreated by extract, the number of cells decreased by two times on the third day of growth. However, in the cell line treated with mycelial extract, cell growth was fully inhibited for 72 hours.

Table 1. Oncological tissue of HeLa cells treated with an intracellular extract of a mycelial culture of the oyster mushroom.

Length of incubation	**Cells number on 0.01 mm^2**		**Mitosis**			
			Control		With mc extract	
	48	72	48	72	48	72
HeLa	33.8±0.8	24.0±0.9	1.82±0.2	0.91±0.1	0.29±0.1	0

Morphological changes in the nuclei in the cell, associated with their destruction, correlate with the neoplastic transformation of tissues. Transformed cells are usually characterized by aneuploidy nuclei, which demonstrate a high degree of variability in size and shape, and their number. In our studies of the anticancer activity of oyster mushroom extract, data on the nature of proliferation and transcription of known malignant tissue lines were used to study the effect of the mushroom culture extract on cytophotometric and morphometric parameters of each tissue studied.

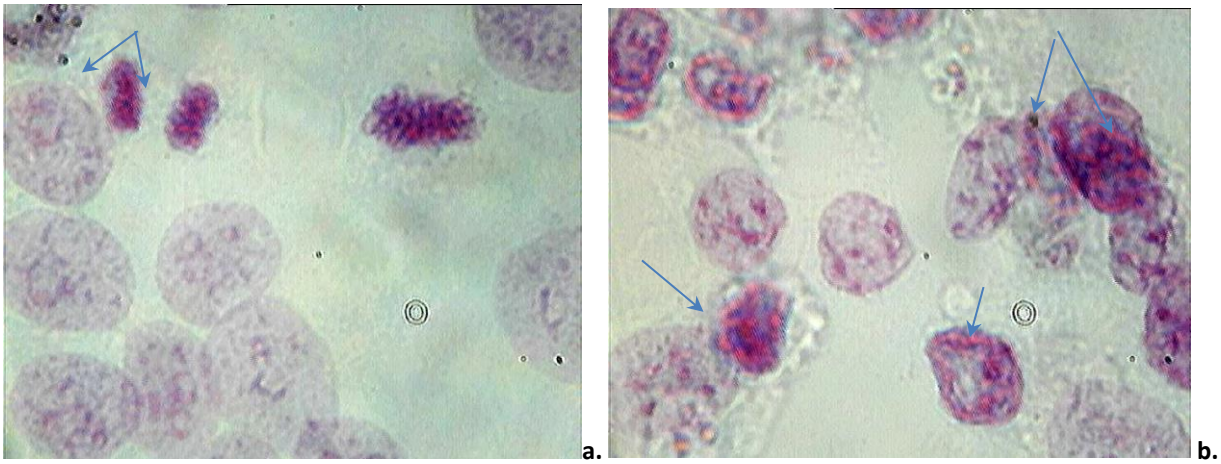


Fig.3 Control preparation of HeLa cells (a), where a mitotic cell is visible in the division phase, and (b) oncological tissue of HeLa cells treated with a mycelial extract of the oyster mushroom.

As our data indicate, extracts of the fungal culture *P. ostreatus* quite sensitively suppress the growth of human tumor tissue cultures: they have an inhibitory effect already at the 48th hour of cultivation and almost completely suppressed the mitotic activity of all studied human tumor cultures by the 3rd day. Morphological changes in the nuclei of cancer cells were associated with loss of proliferative activity, inhibition of growth and differentiation, and damage to cell membranes was observed, which ultimately led to the destruction of tumor cells. In addition to the antiproliferative effect, as observed in our experiments (Fig.3), oyster mushroom extracts affected the membranes of cancer cells, leading to cell clastogenesis, which is possible due to the active action of perforins, having a cytolytic effect on the membranes of cancer cells [28]. The combination of such responses to fungal extracts, in association with different subsets of body cells, may provide greater tumor inhibition than would be expected from exposure to polysaccharides alone, which is also consistent with the literature [6, 7, 29]. Figure 3, b shows cells characterized by aneuploidy nuclei, where the cells have abnormal numbers and structures, suggesting that oncogenes contribute to aneuploidy. A recent study has shown that cancer cells exposed to certain chemotherapeutic drugs tend to have an increased frequency of polyploidy, which is closely associated with increased metastasis, chemotherapy resistance, and recurrence of malignancies [30], all of which explains the interest in cancer-associated polyploidy regions and the development of more effective antitumor drugs.

In the human body, the immune system is responsible for malignant and benign tumor diseases with the help of three types of cells: macrophages, natural killer cells (NK cells), and cytotoxic T lymphocytes (CTL). Cancer patients have suppression of all three of these types of cells in their body, the life span of these cells is short and most importantly, their antitumor activity is at a low level. In all likelihood, the combination of these factors leads to the destruction of the body's antitumor shield [31].

Unlike other wood-decaying mushrooms that we have studied, such as the reishi fungus and shiitake, the oyster mushroom is distinguished by its high content of proteins, vitamins (especially B 12), and minerals and, of course, low content of carbohydrates and sugars, and practically no cholesterol [24, 32]. Therefore, we interpret the therapeutic properties of oyster mushrooms primarily by the content of proteins and antioxidant enzymes, such as lignolytics. Electrophoretic separation of proteins of mycelial extracts in PAGE showed that irradiation by mm-waves changes the

quantitative content of individual fractions [17]. We assume that fractions corresponding to molecular masses of 43 kDa and 15 kDa may be responsible for anti-inflammatory and antitumor activity since these fractions increase when treated with a frequency of 50.3 GHz, which corresponds to the highest anti-inflammatory activity of mycelial extract. As it is known, the molecular weight of universal (hybrid) peroxidases corresponds to 42-45 kDa, and MnP – 38-62.5 kDa, depending on the type of fungus [32]. It should also be noted that the research data of the authors [33] on the quantitative and qualitative study of protein extracts of *P. ostreatus* and their anticancer activity corresponds to our data in the control extract on the quantity and molecular weights of protein electrophoretic fractions in PAAG [34].

Next, analyses were conducted to estimation of the composition of amino acids in the acid-soluble proteins of the mushroom mycelial culture extract, processed at this frequency using high-resolution liquid chromatography (HPLC), was carried out. Judging by the chromatogram, the mushroom extract contains large quantities of aspartic acid, glutamic acid, and other amino acids, as evidenced by the data summarized in Table 2. Since in previous studies, the most pronounced anti-inflammatory activity of intraperitoneal administration was obtained when rats were treated with mycelial culture extract of oyster mushroom, treated with 50.3 GHz mm-waves, therefore, we determined the amino acid composition of acid-soluble proteins from extracts of the mycelial culture of the fungus treated at this frequency. Have been provided the estimated mycelial culture extract composition with two expositions of mm-wave of this frequency: during 20 min and 40 min.

As shown in data of Table 2, in extracts of mycelial cultures of the mushrooms, processed by frequency 50.3 GHz during 20 min there is a significant increase in glutamic acid × 25 times. Have been obtained an increase of the amount of aspartic acid, has increased by 3 times. In the extract such amino acids as glycine and alanine increase by two times, but the amount of serine remains unchanged. The protein of *P. ostreatus* is characterized by a high content of amino acids that are not produced by the human body and must be supplied in large quantities with food. Among such amino acids in these mushrooms are leucine, lysine, and phenylalanine, as presented in the article [34].

Table 2. Amino acid composition of acid-soluble proteins of P. ostreatus mycelial culture extract, in mg/ml.

Amino acids	**Control**	**Treatment**	
		50.3 GHz 40’	50.3 GHz 20’
Aspartic acid	0.04	0.08	0.11
Glutamic acid	0.01	0.15	0.25
Glycine	0.01	0.02	0.02
Alanine	0.05	0.09	0.1
Serine	0.1	0.1	0.1

Thus, aqueous extracts of the mycelial culture of oyster mushrooms are rich in proteins, especially in antioxidants. When exposed to mm waves on a culture of the oyster mushroom, lipid peroxidation, the formation of nano-quantities of peroxides and other reactive oxygen species, most likely occurs, which causes

activation of the defense system of antioxidant enzymes - peroxidases. At the same time, it should be noted that the fungus contains poly-functional peroxidases the active center of which includes glutamic and aspartic acids (both with a negative charge) [33], then an increase in the content of these aminoacids may indicate an increase in the amount of poly-functional peroxidases in the extracted content under the influence of mm waves.

Wood-destroying fungi *P. ostreatus*, or as it is also called white rot fungi, are considered the best organisms for lignin breakdown due to a highly efficient enzymatic system that has hydrolytic and oxidative enzyme systems for lignocellulose degradation, such as a polyfunctional peroxidase, which we activate by treating the mycelial culture with mm-waves. Increased peroxidase activity, in turn, leads to lignin degradation with the release of phenylpropanoids and polyphenols, which most strongly influence the immunomodulatory effects of intracellular protein extracts of mushrooms, especially oyster mushroom extracts [25, 34].

It should be noted that many enzymes of fungal origin are used in various industrial technologies, such as food production, fermentation, and conversion of biomass into ethanol. Only wood-destroying white rot fungi can decompose lignin completely up to obtaining CO_2 and H_2O. From this point of view, *P. ostreatus* is a multifunctional product, since in addition to the rich content of nutritional and biologically active compounds, it is also an important source of enzymes for food and juice processing.

CONCLUSIONS

The mushrooms are rich in essential proteins, considered to cover the dietetic requirements in comparison to plant proteins, which lack some essential amino acids [**35**]. The high content of such amino acids as glutamic acid, aspartic acid, and sulfur amino acid (methionine and cysteine) contents contribute to the meaty taste of processed foods and thus may serve as flavor enhancers [36]. Among them, mushroom powder has been used as a meat replacement in chicken nuggets and beef salami; as a partial wheat flour replacement in biscuits; and as a substrate to produce novelty alcoholic drinks with unique flavors. Additionally, with the surge in the incidence of cardiovascular disease, type-2 diabetes, and cancer, there is a need to develop new dietary strategies and to develop foods that could potentially support disease prevention [37]. *Pleurotus* spp. are becoming increasingly attractive as row source for the development of new drugs and functional foods due to their potential antioxidant, antimicrobial, anti-proliferative, immunomodulatory, anti-inflammatory, anticancer and anti-hypertensive properties [3-5, 8, 34].

The fact that HPV can lead to cervical cancer paves the way for development of one of the first anti-cancer vaccines. However, the above vaccines do not treat existing HPV infections or diseases caused by HPV. These vaccines work by stimulating the immune system to specifically target and kill infected cells. Since today researchers are working to develop therapeutic HPV vaccines, which instead of preventing HPV infection would prevent cancer from developing among women previously infected with HPV [3], and our research aims to seek new and more effective medicinal products for treating such heavy and mortal diseases.

Our studies have shown the high anti-inflammatory activity of injections of aqueous extract of the mycelial culture of *P. ostreatus* [17-19], which will allow us to treat a wide range of inflammatory processes in the body. We evaluated the cytotoxic and cell death induction effects on water-soluble extracts of *P. ostreatus* mycelium in the cervical cancer cell lines HeLa (HPV18) [38-39]. Our early obtained data is now confirmed by the minor number of papers [4-5]. The white rot mushroom extract, in addition to carbohydrates, proteins, nucleic acids, lipids, minerals, and vitamins [6, 20, 24], after mm-wave treatment, was enriched with a high content of aspartic and glutamic

acid, which are key amino acids in the active center of the polyfunctional peroxidase of *P. ostreatus* [33], and, as we testify, it is the polyfunctional peroxidases that play a key therapeutic role in the mycelial extract of the mushroom.

As a result, the complex of proteins and enzymes in the oyster mushroom extract, enriched with neuromodulator amino acids, becomes a powerful tool in the fight against neoplastic transformations in human cells [38, 39] According to the data, an important role is assumed for hybrid fungal peroxidases, primarily candidates for a biologically active substance in the mycelial culture extract, acting as anti-carcinogenic, anti-inflammatory and immunomodulatory agents.

In conclusion, it should be noted that such functional food as edible mushrooms *P. ostreatus*, which contain many proteins, especially poly-functional peroxidases, and have immunomodulatory properties, can provide the human body with proteins and many biologically active compounds, which, in bonus, act as strong pharmaceutics for many diseases, including various human cancers. Future investigations of the extract's effect on cells will be tested on 3D spheroid models. The most effective extract will be further studied *in vivo* in rats, pathing the way for creating a potential drug to combat certain types of cancer.

List of abbreviation: HPV – human papilloma virus, HPLC - high-resolution liquid chromatography, mm-waves-electromagnetic wave in the range of 300 MHz–300 GHz frequencies.

Author Contributions: Conceptualization, Methodology, Formal Analysis, Investigation, Resources, Data Curation, Funding Acquisition: I.A.A. and L.A.M.; Writing, Review and Editing: I.A.A. and N.S.B Project administration, Supervision, Writing, Original Draft Preparation: L.A.M.

Conflicts of interest: The authors declare no conflict of interest relevant to this work.

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All authors have read and agreed to the published version of the manuscript.

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