After that, the morning or evening melatonin administrations were given to normal and obese animals at a dose of 30 mg/kg for 7 weeks. Under conditions of the spring-autumn photoperiod (12L:12D) was studied in this work. The obesity was caused with a high-calorie diet for 6 weeks. Melatonin (both morning and evening) to animals without obesity causes an activation of the mucosa, hypertrophy of goblet cells, reduction of structural-functional changes in the large intestine during obesity by melatonin. Although, the morning administration of melatonin had some normalizing effects on the colon and it was more effective than evening administration.

Structural changes in the colon of rats with obesity and its correction by morning and evening injection of melatonin

The effect of morning and evening administrations of melatonin on structural and functional changes in the large intestine of rats with obesity under conditions of the spring-autumn photoperiod (12L:12D) was studied in this work. The obesity was caused with a high-calorie diet for 6 weeks. After that, the morning or evening melatonin administrations were given to normal and obese animals at a dose of 30 mg/kg for 7 weeks. After 13 weeks, two specimens of the colon 1 cm each were taken at a distance of 3 cm from the anus; fixed in 10% formalin and in Carna solution; paraffin sections of the large intestine were made; stained them with hematoxylin-eosin, alcian blue-carmine, or toluidine blue. Microscopic and morphometric analysis of these sections was performed. It has been shown, that obesity cause hypervascularization of the colonic mucosa, reduction of colonic crypts, hypertrophy of goblet cells and overaccumulation of granules in mast cells. Morning administration of melatonin to obese animals normalizes the colonic mucosa, decreases the reduction of colonic crypts, but causes the hypotrophy of goblet cells. Evening administration of melatonin significantly decreases the reduction of colonic crypts, but does not eliminate other changes caused by obesity. The administration of melatonin (both morning and evening) to animals without obesity causes an activation of the mucosa, hypertrophy of goblet cells, reduction of colonic crypts, and does not change the state of mast cells. Consequently, it cannot make a clear conclusion about the possibility of correction of all structural-functional changes in the large intestine during obesity by melatonin. Although, the morning administration of melatonin had some normalizing effects on the colon and it was more effective than evening administration.

Key words: melatonin, obesity, large intestine.

Effect of bioactive extracts with high cytokinins content from micelial biomass of hericium coralloides and fromomopsis officinalis on tumor cells in vitro

Phytohormones cytokinins are known to promote cell division in plants. Contrary, in animal’s and human’s tissues they induce apoptosis and block the cell cycle of a wide spectrum of tumour cells. Therapeutic effects of cytokinins, specifically their anticancer and immunomodulatory actions are similar to those of medicinal mushrooms. We detected cytokinins in mycelial biomass of two species of medicinal mushrooms growing in vitro (Fomitopsis officinalis strain 5004 and Hericium coralloides strain 2332) using HPLC-MS. Trans-zeatin, zeatin riboside, zeatin-O-glucoside and isopentenyladenine were found. Crude extracts and purified cytokinin fractions from mycelial biomass were tested on the growth and development of cultures of tumor cells lines: Hela (MTT-assay), T24/83 (viability and level apoptotic cells) and HepG2 (consumption of glucose). The effect of cytokinin fraction from mycelial biomass of Fomitopsis officinalis on pathogenic cells was higher compared to Hericium coralloides one. The data obtained revealed a higher cytotoxic/cytostatic effect of the purified cytokinin fractions in comparison with crude methanolic extracts; also higher apoptotic index was found. Under the influence of the test agents the intensification of glucose uptake into cells was observed. This indicator was higher for crude mushroom mycelium extracts, whereas under the action of purified fractions the glucose uptake rate was lower, thus decreased glycolysis level was recorded. Also, the effect of both crude extract and purified fraction from H. coralloides mycelial biomass on glucose uptake in the conditioned medium was lower against F. officinalis. These results confirm the assumption that biologically active substances of medicinal mushrooms with high pharmacological potential include cytokinins.

Key words: medicinal mushrooms, mycelial biomass, cytokinins, HepG2, T-24/83 and Hela cells.

Introduction. Deadly side effects of artificially synthesized drugs can be avoided only by means of natural preparations or those that are as close as possible to them in composition and structure of substances. Therefore, the urgent task of today is to find alternative therapies using natural biological raw materials. Macromycetes have enormous potential in this regard. Medicinal mushrooms have a wide range of medicinal properties. They exhibit more than 130 therapeutic effects due to the content of biologically active substances in fruiting bodies and cultured mycelium that enhance innate and acquired immune responses and demonstrate antitumor activity in animals and humans [1, 2]. Polysaccharides and terpenoids are the most studied among them. However, since the systematic study of the biochemical composition of mushrooms, the physiological and medical action of its components has
begun recently, the list of such compounds is not comprehensive. Most likely, the medicinal properties of mushrooms are determined by the presence of a complex of compounds that act jointly, enhancing the effect of each other [3]. The active substances of medicinal mushrooms probably include phytohormones, in particular, cytokinins, whose therapeutic effect has been detected in the last decade [4]. Cytokinins are polyfunctional hormones of plants that are involved in regulating their growth and development in many aspects, in particular, positively regulating cell division [5]. At the same time, in animal cell culture the addition of cytokinins is known to have an opposite effect. Thus, cytokinin analogues have been found to block cell cycle patern and inhibit the growth of many types of human cancers [6, 7]. The therapeutic properties of cytokinins were similar to those shown by medicinal mushrooms. The ability to produce cytokinins is inherent in both fruiting bodies [8] and mycelial culture [9] of many medicinal mushroom species. Therefore, it can be assumed that the medicinal properties of mushrooms depend on the cytokinins synthesized in their cells in combination with fungal specific metabolites. However, medical cytokinins testing has only recently begun, and detailed information is lacking today. Species of basidiomycetes, which have been used in traditional Chinese and European medicine for many centuries, include the honeysuckle coral-like *Hericium coralloides* and the larch sponge *Fomitopsis officinalis* [10]. These are tree-destructive species with a large fruiting body that cause wood rot. In *H. coralloides*, they contain a complex of substances that are used as antidepressants, antioxidants, in the treatment of Alzheimer’s and Parkinson’s diseases and a number of cancers, to fight insomnia and impotence, to reduce blood cholesterol, etc. [11]. Extracts from *F. officinalis* fruiting body have a wide range of antimicrobial and antiviral activity due to the content of coumarins and triterpenoids, but the crude extract exhibits the highest activity, in particular against *Mycobacterium tuberculosis* and *Yersinia pseudotuberculosis* [12].

A necessary step to determine the nature of the biologically active substances of medicinal mushrooms is to investigate the effect of cytokinins produced by them on the growth and development of pathogenic cells.

**Materials and Methods.**

1. **Micelial biomass cultivation**

*Hericium coralloides* (Scop.) Pers., strain 2332, and *Fomitopsis officinalis* (Vill.) Bondartsev & Singer, strain 5004, from the IBK Mushroom Culture Collection were studied (Fig. 1). Fungi strains were cultivated in 250 ml Erlenmeyer flasks with 50 ml medium in stationary conditions (26 ± 1 °C) during 20 days in darkness. Inoculation with physiologically active mycelium in proportion of 10 % to the total volume was carried out in accordance with the method developed for Basidiomycetes [13]. Microbiological control of nutrient medium and inoculum material purity was fulfilled before inoculation. For cultivation of *F. officinalis* 5004 the following liquid nutrient medium was used: glucose – 30.0 g/l; NH₄NO₃ – 3.5 g/l; KCl – 0.5 g/l; KH₂PO₄ – 1.0 g/l; MgSO₄·7H₂O – 0.5 g/l; beer wort (15 ° in accordance with Baling method) – 115 ml; pH 5.0. For cultivation of *H. coralloides* 2332 such liquid nutrient medium was used: glucose – 25.0 g/l; peptone – 3.0 g/l; yeast extract – 3.0 g/l; KH₂PO₄ – 1.0 g/l; K₂HPO₄ – 1.0 g/l; MgSO₄·7H₂O – 0.25 g/l; pH 6.5. Media acidity was maintained at necessary pH levels by 1 N KOH and 1 N HCl solutions addition.

Micelial biomass was separated from the culture medium through filtration under vacuum followed by a double rinsing with 50 ml of potassium-phosphate buffered saline, pH 6.5.

![Fig. 1. Fruiting bodies of *Hericium coralloides* (left) and *Fomitopsis officinalis* (right) in nature](image)

2. **Cytokinins purification**

The sample (10 g of micelial biomass) was homogenized during 3 min using an electrical homogenizer (Mechanika Precyzyjna, Poland) in 80 % methanol solution. Cytokinins were extracted with 80 % methanol (10 ml per 1 g) thrice during 24 h at +4°C. The obtained extract was evaporated under vacuum using the rotor evaporator (Unipan, Poland) at +50 °C to a water phase state. Water was evaporated under vacuum using the rotor evaporator on column 20х2 cm (Bio-Rad, USA) with Dowex 50WX8 (Serva, Germany) in H⁺ form, elution with 0.1 N ammonia. Eluate was evaporated under vacuum to a dry residue, which was dissolved in 1 ml of 96 % ethanol and applied on thin layer chromatography plates Silicagel 60 F254 (Merk, Germany), run in solvent system isopropanol:ammonia:water (10:1:1 by volume).

3. **HPLC/MS analysis**

Detection and quantification of cytokinins were performed using the HPLC-MS system (Agilent 1200, USA). Solid samples were dissolved in 200 μl of mobile phase and 5μl aliquot was injected into Agilent Zorbax Eclipse XDB-C18 column (4.6x250 mm, 5 μm). The column was eluted with an isocratic solvents system methanol:water:acetic acid (37:62:9.0:1 by volume) at a flow rate of 0.5 ml/min and column temperature of +30°C. The fractions eluted were directly passed through the mass spectrometer (Agilent 6120 Quadrupole LC/MS) in a combined regime “multi mode” (electrospray and chemical ionization at atmosphere pressure) of positive ionization. Data were analysed and processed using the software Agilent Chem-
The cytokinin hormone content in mycelial biomass of medicinal mushrooms, ng/g FW

| Mushrooms species                  | t-Z*       | ZR*       | iPa*       | iP*       | ZOG*       | Σ          |
|-----------------------------------|------------|-----------|------------|-----------|------------|------------|
| Hericium coralloides, strain 2332| 941,12±46,8| 531,99±26,1| 0          | 348,60±17,3| 0          | 2044,47    |
| Fomitopsis officinalis, strain 5004| 81,41±3,7  | 146,21±6,7| 0          | 0         | 167,34±7,5| 525,41     |

Notes: t-Z* - trans-zeatin, ZR* - zeatin riboside; iPa* - isopentenyladenosine, iP* - isopen
tenyladenine, ZOG* - zeatin-O-glucoside

Modern science considers mushrooms as producers of a wide range of compounds that can affect multiple processes in the human body synergistically, so it is important to study the combinations of molecules in fungal extracts [17]. We have established the ability of mycelial biomass of two species of fungi with a high pharmacological potential to produce cytokinins in large quantities. Comparison of the spectra of the pharmacological properties of medicinal mushrooms and cytokinins suggests that cytokinins are one of the components providing the therapeutic effect of macromycetes. In this regard, cytokinin-containing mycelial biomass fractions of H. coralloides and F. officinalis were tested on cultures tumor cells lines: Hela (MTT-assay), T24/83 (viability and level apoptotic cells) and HepG2 (consumption of glucose) The crude methanol extracts and cytokinin fractions, purified to the stage of ion exchange chromatography inclusive, were investigated.

The cytotoxic/cytostatic or proliferative effect (cell viability) was estimated as a percent of live cells in comparison against control and expressed in term of median growth inhibition (GI50), the compound’s concentration that causes 50% decrease in the net cell growth or mitogenic stimulation vs control). Higher activity was found for purified cytokinin fractions than for crude methanol extracts. The inhibitory effect for both mushroom species after cytokinin fractions treatment was in the range of 0.04-0.08 mg/ml, whereas IC50 was not determined for crude methanol extract due to large deviations in parallel measurements (Fig 2 and Fig 3).
Fig. 2. Cytotoxic/cytostatic influence of *F. officinalis* cytokinin fractions (1) and crude methanol extract (2).

Typical photo MTT-test

Fig. 3. Cytotoxic/cytostatic influence of *H. coralloides* cytokinin fractions (1) and crude methanol extract (2).

Typical photo MTT-test
To evaluate the level of apoptotic cells and percent viability the cells line of bladder tumor T24/83 was used. The content of dead cells under the effect of the crude extracts was 18,7±4,3% for *H. coralloides* and 24,3±3,3% for *F. officinalis*, respectively, while it was 7,6±1,4% and 10,2±3,4% for the purified fractions for *H. coralloides* for *F. officinalis*, respectively. However, the percentage of apoptotic cells was higher for the purified fractions with the addition of equimolar concentrations of the substances: 32,8±5,6 for *H. coralloides* and 34,4±1,3 for *F. officinalis*, respectively, while for crude extracts of *H. coralloides* and *F. officinalis* these values were 27,0±1,6 and 23,4±2,8, respectively.

For metabolic pathways studies we used usually Hep G2 cells. As can be seen from the data under the influence of the test agents the intensification of glucose uptake into cells was observed. This indicator was higher for crude mushroom mycelial extracts, whereas under the action of purified fractions the glucose uptake rate was lower (Table 2). The effect of both crude extract and purified fraction from *H. coralloides* mycelial biomass on glucose uptake in the conditioned medium was lower against *F. officinalis*.

**Table 2.** The effect of extracts from *Hericium coralloides* and *Fomitopsis officinalis* mycelial biomass on consumption of glucose by HepG2 cells; (M ± m, n = 5, * – p < 0.05 relative to control ^-relative crude extract)

| Test agent             | Glucose level (mM) in conditional medium |
|------------------------|------------------------------------------|
| Control                | 5,9±0,3                                  |
| Hericium coralloides crude extract | 1,9±0,1*                                 |
| Hericium coralloides purified fractions | 2,4±0,2**                               |
| Fomitopsis officinalis crude extract    | 3,2±1,0*                                |
| Hericium coralloides purified fractions | 4,3±0,3**                               |

Cytokinins are known to change morphology and disorganize actin cytoskeleton of bladder carcinoma T24 cells, block DNA synthesis and increase the level of cycline-dependent kinase inhibitor and induce genes involved in a negative regulation of the cell cycle in tumour cells of epithelium [18–20]. Cytokinins also inhibit the human enterovirus replication, show immunostimulating effects promoting proliferation of natural killer cells, provoke apoptosis in myeloid leukemia HL-60 cells [21–23]. Thus, comparing the data obtained with the literature, we can reasonably assume that the inhibitory effect of fungal extracts on tumor cells is associated with the presence of cytokinins in them. Moreover, the cytokinin fraction from the *F. officinalis* mycelial biomass exhibited greater activity compared to *H. coralloides* one despite the higher cytokinin concentration in it. It has previously been found that aqueous and ethanol fungal extracts from *F. officinalis* exhibit growth-inhibition effect on different cancer cell lines (mouse sarcoma, human hepatoma, lung cancer, colon cancer and breast cancer) [24], whereas *H. coralloides* has another medicinal properties. Perhaps, cytokinins act in complex with different compounds in these mushroom species.

**Conclusion.** In the present study the influence of extracts from two species of medicinal Basidiomycetes mycelial biomass on tumor cells of different origin was examined. The data obtained revealed a higher cytotoxic/cytostatic effect of the purified cytokinin fractions in comparison with crude methanolic extracts; also higher apoptotic index and decreased glycolysis were recorded. These results confirm the assumption that biologically active substances of medicinal mushrooms with high pharmacological potential include cytokinins.

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ВЛАЯНЯ БІОЛОГІЧЕСКИ АКТИВНИХ ЕКСТРАКТОВ
С ВИСОКИМ СОДЕРЖАНИЕМ ЦИТОКИНИНОВ С МИЦЕЛІАЛЬНОЮ БІОМАССОЮ HERICUM CORALLOIDES
І FOMITOPSIS OFFICINALIS НА ОПУХОЛЕВІ КЛЕТКИ IN VITRO

Известно, что фитогормоны цитокинины стимулируют деление клеток у растений. И напротив, в юных животных и человека они индуцируют apoptosis и блокируют клеточный цикл широкого спектра опухолевых клеток. Терапевтическое действие цитокини
нов, в частности их противоповреждающее и иммуномодулирующее действие, аналогично действию лекарственных грибов. Мы обна
ружили цитокинины в микелиальной биомассе двух видов лекарственных грибов, выращенной in vitro (Fomitopsis officinalis штамм 5004 и Hericium coralloides штамм 2332). Выявлено транс-замен, заметрибизид, заметон-α-глюказид и изолептинидин. Исследо
вали влияние неочищенных экстрактов и очищенных фракций цитокининов из микелиальной биомассы на рост и развитие культур линий опухолевых клеток: HepG2 (MTT-анализ), Т24 / 83 (кислотностойкость и уровень апоптотических клеток) и HepG2 (усиление глю
козы). Выявлено более высокое цитотоксическое / цитостатическое действие очищенных фракций цитокининов по сравнению с неочищенными метанольными экстрактами; также апоптотический индекс был зафиксирован выше. Под влиянием исследуемых экстрактов наблюдалось усиление появления глюкозы в культуре. Это подтверждается тенденцией, что в составе биологически активных веществ заростков лекарственных грибов с высоким фармакологическим потенциалом входят цитокинины. Ключевые слова: лекарственные грибы, микелиальная биомassa, цитокинины, HepG2, Т24-83 и Нелла клетки.

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ERECHITITES HIERACIFOLIA (L.) RAF. EX DC. (ASTERACEAE BERTHC. & J. PRESL) – НОВИЙ АДЕВТЕНТИВНИЙ РОСЛИНІЙ ДЛЯ ФЛОРИ КИЇВСЬКОГО ПОЛІСЯ

Повидомлено про знахідку Erechites hieracifolia (L.) Raf. ex DC. (Asteraceae Bercht. & J. Presl) – нового виду адевенті
вних рослин для флори Київського Полісся, зафіксованого на території Чорнобильського радіаційно-екологічного біо
сферного заповідника та Національного природного парку "Голосіївський". Вид північноамериканського походження, за часом занесення – кенофіт, за способом занесення – кенофіт, за ступенем натурализації – колонофіт. Уперше в регіоні досліджень його виявлено в 2018 р. на території біосферного заповідника: на північній околиці Київського о. Іншими місцеми заростів відомі у долині р. Десна, у Київській області.

Вступ. Останнім часом в Україні спостерігається ак
тивне поширення низки видів адевентивних рослин, зок
кроме і тих, що належать до групи інвазивних. Врахову
ючи їхніх негативних вплив на довкілля, такі види по
требують моніторингу та розробки заходів контролю за
їхнім подальшим розповсюдженням. До таких, зокрім,
належить Erechites hieracifolia (L.) Raf. ex DC. (Aster
aceae Bercht. & J. Presl), вид північноамериканського
походження [6]. Рослини виду продукує велику кіль
кість (до 30 000) насіння з однієї рослини [14, 16], яке
зберігає здатність до проростання протягом всього років [13]. Утворюючи масові зарості, E. hieracifolia, зо
крема в Поліському природному заповіднику, на частині післепожжених ділянок щільність його популяції скла
дає 60–80 особин/м², що призводить до збіднення фло
ру, трансформації природних угруповань та подальшого розвитку агрономічного загрози, яка визване незгідніс
чома та повною замінує одні типи рослин або видов
ними. На ділянках, де відбувається розповсюдження виду, відбувається загроза руйнування природних угруповань, навіть забруднення відходами і забрудненням від активного поширення інших рослин. На сьогодні відбуваються природно-гідроекологічні процеси та природні розповсюдження виду в Україні, який пошириється з усіх східних та природних регіонів України, відкриваючи тенденцію до активного поширення в інші регіони. В Європі відбувається відкритої інвазії видів, яка підлягає контролю з метою забезпечення безпечного розвитку рослин.

Ключова слова: Erechites hieracifolia, вид адевентивних рослин, флористична знахідка, Київське Полісся, Україна.