Exp Oncol 2014
36, 2, 58–66

REVIEW

Experimental ONCOLOGY

ANTICANCER SUBSTANCES OF MUSHROOM ORIGIN

T.S. Ivanova[1], T.A. Krupodorova[1], V.Y. Barshteyn[1,], A.B. Artamonova[2], V.A. Shlyakhovenko[2]*

[1]Institute of Food Biotechnology and Genomics of National Academy of Sciences of Ukraine, Kyiv 04123, Ukraine

[2] R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, National Academy of Sciences of Ukraine, Kyiv 03022, Ukraine

The present status of investigations about the anticancer activity which is inherent to medicinal mushrooms, as well as their biomedical potential and future prospects are discussed. Mushroom products and extracts possess promising immunomodulating and anticancer effects, so the main biologically active substances of mushrooms responsible for immunomodulation and direct cytotoxicity toward cancer cell lines (including rarely mentioned groups of anticancer mushroom proteins), and the mechanisms of their antitumor action were analyzed. The existing to date clinical trials of mushroom substances are mentioned. Mushroom anticancer extracts, obtained by the different solvents, are outlined. Modern approaches of cancer treatment with implication of mushroom products, including DNA vaccinotherapy with mushroom immunomodulatory adjuvants, creation of prodrugs with mushroom lectins that can recognize glycoconjugates on the cancer cell surface, development of nanovectors etc. are discussed. The future prospects of mushroom anticancer substances application, including chemical modification of polysaccharides and terpenoids, gene engineering of proteins, and implementation of vaccines are reviewed.

Key Words: **mushrooms, anticancer substances, extracts, vaccinotherapy.**

INTRODUCTION

The most recent approaches in cancer treatment include construction of nanovectors for drug-delivery and diagnostics [1], design of prodrugs that can be converted to active form when targeting tumor cells [2], gene therapy [3], and other methods which have an impact on malignant cells but don't affect normal cells. These technologies are more effective in the early stages of the disease, and the best result is achieved when combined with classical chemotherapy and radiotherapy [3]. Thus, chemotherapy counts more than 100 anticancer drugs with different mechanism of action. Numerous side effects of chemo- and radiotherapy require the use of additional remedies to maintain and improve the immune status of patients. For instance, anticancer therapy can include the use of herbal drugs, especially those that have cytotoxic properties with minimal side effects. Mushrooms attract more and more interest as a raw material for the production of such drugs. Therapeutic properties of medicinal mushrooms were exploited by folk medicine throughout the world since ancient times [4]. Nowadays about 14 thousand of mushroom species are known [5]. There are different data about the number of mushroom species possessing anticancer activity: from 200 to 331 (out of 540 mushroom species which are used in China medicinal practice) [6]. After analyzing of these mushroom species one can say that 150 of them are inherent to Ukraine.

Numerous investigators appealed to *Macromycetes* in urge to find efficient herbal remedies for anticancer therapy. So, the determination of the present status of investigations about the anticancer activity of medicinal mushrooms, their biomedical potential and future prospects of usage are important and urgent.

ANTICANCER SUBSTANCES OF MUSHROOM ORIGIN

Biologically active substances of medicinal mushrooms with anticancer action comprise polysaccharides, polysaccharide-protein complexes, dietary fiber, certain types of proteins, terpenoids, steroids, phenols, etc.

Submitted: March 20, 2014.

*Correspondence: Fax: +38 044 462-72-59
E-mail: ihtbar@rambler.ru

Abbreviations used: Bcl-X(L) – B-cell lymphoma (extra-large) transmembrane molecule in the mitochondria; ED_{50} – effective dose for 50% of the group; EGF – epidermal growth factor; Fas/APO-1 – apoptosis antigen 1; GTP – guanosine triphosphate; HMAF – 6-hydroxymethylacylfulvene; HPV – human papillomavirus; IC_{50} – half maximal inhibitory concentration; IFN – interferon; IL – interleukin; IκBα – nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha; NF-κB – nuclear factor kappa-light-chain-enhancer of activated B cells; PSK – polysaccharide K; PSP – polysaccharide peptide; TGF – transforming growth factor; TNF – tumor necrosis factor; T_h – T helper cells.

Cell lines and tumor strains: A549 – human lung epithelial carcinoma; Bre-04 – human breast carcinoma; B16 – murine melanoma; CAL27 – human oral cancer; CCD 841 CoTr – normal human colon epithelium; CH72 – murine skin carcinoma; colon 26-L5 – murine liver metastatic carcinoma; C50 – normal mouse skin keratinocytes; Ehrlich's carcinoma – mouse breast carcinoma; HeLa – human cervical cancer; Hep G2 – human liver carcinoma; HL-60 – human promyelocytic leukemia; HPAF-II – human pancreatic adenocarcinoma; HTC – rat liver carcinoma; HT-29 – human colon adenocarcinoma; HT-1080 – human fibrosarcoma; Huh-7 – human hepatoma; Jurkat – human T cell leukemia; K562 – human myelogenous erythroleukemia; LLC – Lewis lung carcinoma; LS180 – human colon adenocarcinoma; Lu-04 – human lung carcinoma; L1210 – mouse lymphocytic leukemia; MCF-7 – human breast adenocarcinoma; MDA-MB – human mammary gland adenocarcinoma; MV522 – human lung carcinoma; MX-1 – human ductal breast adenocarcinoma; PC-3 – human prostate cancer; PLC/PRF/5 – (Alexander) human hepatoma; PL45 – human pancreatic epithelial adenocarcinoma; P388 – murine lymphocytic leukemia; RPMI-8226 – human myeloma; T24 – human urinary bladder carcinoma; T-47D – human ductal breast epithelial tumor; Vero – monkey kidney normal cells; WI-38 – human lung fibroblast; WM-1341 – human melanoma.

Polysaccharides and polysaccharide-protein complexes

Biologically active polysaccharides were detected in fruiting bodies, mycelial mass and cultural broth of *Macromycetes*. Mushroom polysaccharides prevent carcinogenesis and metastasis, and, in addition, display immune cell-mediated anticancer activity [7]. Polysaccharides with antitumor activity belong predominantly to glucans with β-(1→3) bonds in the main chain and with β-(1→3) bond side branches, wherein side branches are essential for occurrence of biological activity. Furthermore, it was demonstrated [8] that high-molecular weight polysaccharides are more efficient than low-molecular. It is generally recognized that application of mushroom polysaccharides can be an additional remedy for cancer patient's treatment, but it is reasonable to combine such agents with traditional methods of therapy like surgery, chemo- and radiotherapy [3]. In such combination polysaccharides, polysaccharide-protein complexes, and mushroom extracts have an ability to reduce side effects of conventional cancer treatments [8]. Worth to note, that clinical trials of mushroom origin drugs are sufficiently expensive and long-term. Therefore, there are plenty investigations of medicinal mushroom anticancer activity *in vitro* and *in vivo*, but, to the best of our knowledge, read by the number polysaccharides and polysaccharide-protein complexes have passed clinical trials (Table 1). Mentioned in the Table 1 substances have undergone clinical trials I–III phases in Japan, China, and USA. Initial phases of clinical trials have shown anticancer activity of these drugs and absence of short-term or long-term toxicity, as well [8].

Table 1. Mushroom antitumor polysaccharide and polysaccharide-protein complexes which have passed clinical trials

Taxa	Origin of isolation	Trade name	Chemical structure	Ref.
Grifola frondosa	Fruiting bodies and mycelium	D and MD-fractions	β-D-glucan	[7]
Lentinus edodes	Fruiting bodies	Lentinan	β-D-glucan	[8]
Schizophyllum commune	Cultured broth	Schizophyllan	β-D-glucan	[8]
Trametes versicolor	Mycelial mass	PSP, PSK (Krestin)	Polysaccharide-protein complexes	[8]

Lentinan (see Table 1) is a water-soluble anticancer polysaccharide, which was isolated from fruiting bodies of *L. edodes* by Chihara et al. [9] in 1969. Lentinan has a molecular weight 500 kDa, the main chain consists of glucose, connected with β-(1→3) bonds, and side chains, connected with main chain by β-(1→6) bonds. Lentinan binds to lymphocyte cell surface or to specific proteins of blood serum which activate macrophage, T-killers, and other effector cells that, in turn, enhance antibody, IL-1, IL-2, and IFN-γ production. Lentinan completely inhibits the growth of subcutaneously transplanted in mice Sarcoma-180 [4]. In clinical trials [10] after the administration of tegafur to patients, suffering from gastric cancer, the survival rates increased during the first and second years at the same level (2.9%), though during the third year an increase in survival rate was not observed. Patients who had been treated with tegafur in combination with lentinan, during 1, 2, and 3 years (1 mg twice a week or 2 mg once a week administered intravenously) showed survival rate increases by 19.5; 10.4, and 6.5%, respectively. Similar results were obtained in case of progressive and relapsing rectal cancer [11]. At the same time, administration of lentinan in combination with anticancer drugs mitomycin C and 5-fluorouracil didn't lead to any positive results. If combined lentinan with radiotherapy and surgery, a positive effect was achieved in treatment of lung, breast, and gastric cancer [11]. Adverse reactions and toxicity of lentinan were observed in rare cases [4].

The structure and anticancer activity of schizophyllan (see Table 1) are similar to lentinan [8]. Schizophyllan is a (1→3)-β-glucan in linear linkage with (1→6)-β-glucopyranoside branches, bonded to every third or fourth residue [12]. Schizophyllan restores and enhances cellular immunity in the tumor-bearing host by functioning as a T-cell adjuvant and macrophage activator; it also induces the gene expression of cytokines [12]. In *in vivo* studies [12] schizophyllan inhibits solid Sarcoma 180 and increases survival of mice when injected by intraperitoneal or intravenous route, but has low antitumor activity by subcutaneous route. It also has no effect on the survival of mice bearing Sarcoma 37, Ehrlich's carcinoma, or Yoshida sarcoma [12]. Clinical studies with schizophyllan in combination with conventional chemotherapy (tegafur or mitomycin C and 5-fluorouracil) in a randomized study of 367 patients with recurrent and inoperable gastric cancer resulted in a significant increase in median survival [8]. Recently it has also been shown, that schizophyllan increases overall survival of patients with head and neck cancers [8]. In a randomized study, schizophyllan, in combination with radiotherapy, significantly prolonged the overall survival of stage II cervical cancer patients but not stage III [8]. Schizophyllan is currently produced commercially by several Japanese pharmaceutical companies and approved for clinical use in Japan.

Two different polysaccharide fractions with anticancer properties were obtained from mycelial mass of basidiomycete *T. versicolor.* PSK (krestin) is a β-D-glucan-protein complex which was obtained by Japan company "Kureha Kagaku Koguo K.K." by the method of hot water extraction [13]. Mechanism of krestin anticancer activity involves the following actions: recovery from immunosuppression induced by humoral factors such as TGF-β or as a result of surgery and chemotherapy; activation of antitumor immune responses including maturation of dendritic cells, correction of Th1/Th2 imbalance, promotion of IL-15 production by monocytes; and enhancement of the antitumor effect of chemotherapy by induction of apoptosis and inhibition of metastasis through direct actions on tumor cells [14].

The researchers of the Shanghai University (China) have isolated the substance named PSP (consists of 90% polysaccharide and 10% of protein, molecular weight is about 100 kDa) from mycelial mass

of *T. versicolor* Cov-1 [13]. The protein part is composed of asparagines and glutamic acid, the main component of polysaccharide is glucose in the main chain, which is bonded with α-(1→4) and β-(1→3) glycoside linkages. The chemical structure of PSP is similar to PSK, but the former contains fucose, and the latter — arabinose and rhamnose [15].

970 patients took part in clinical trial of PSP has been performed by Yang et al. [15]. 485 tumor patients were given PSP along with chemo- and radiotherapy in this research; control tests were made of 211 cases; random groups were selected for forward-expecting and double-blind control tests from the remaining 274 cases. Various kinds of cancer were involved in the study: gastric (162 cases), esophagus (172 cases), and primary pathogenic lung cancer (151 cases). Tested group was treated with 3 g of PSP daily over two periods with duration of one month. The number of cases of increased natural killer cell activity, of increased IL-2, and CD^{4+}/CD^{8+} ratio was more than observed in the control group. Addition of PSP lowered side effects of chemo- and radiotherapy, appreciable relief was evident especially in the cases of poor appetite, weakness, tiredness, dryness of mouth and throat, and pain. Quality of tumorous patients' life improved on parameters of body weight and Karnofsky's evaluation.

Polysaccharide fractions of *G. frondosa* were also extensively investigated. D-fraction of hot water deproteinated extract from both the fruiting bodies and mycelia of *G. frondosa* consists predominantly of β-D-glucan with 1→6 bonds in the main chain and 1→4 branches or 1→3 bonds in the main chain and 1→6 branches [8]. Mechanism of D-fraction action includes activation of macrophages, which, in turn, increase the production of cytokines. D-fraction of *G. frondosa* appears to inactivate glyoxalase I in *in vitro* studies, an enzyme believed to metabolize chemotherapeutic compounds used against cancer cells thus potentially enhancing their bioavailability [8]. The MD-fraction has been obtained by further purification of the D-fraction and showed a superior anti-cancer effect. The MD-fraction can induce cancer cell apoptosis *via* activation of the BAK-1 gene [16]. The combination of IFN-α2b and the MD-fraction has been shown to have a synergistic effect that triggers DNA-dependent protein kinase activation and induces cancer cell arrest at the G1 cell cycle checkpoint. The D- and MD-fractions are undergoing phase I/II clinical trials in the United States and Japan [16].

There are some methods of anticancer activity and clinical quality intensification for polysaccharide remedies. Thus, carboxymethylated, hydroxylated, formylmethylated, aminethylated and sulfated products have been designed to improve the biological activity by chemical modification. Particularly, the linear (1→3)-α-glucans from *Amanita muscaria* and *Agrocybe cylindracea* have little antitumor activity, but the carboxymethylated linear (1→3)-α-glucan showed high potent antitumor activity against Sarcoma 180 and immunomodulatory activity in mice [12].

Mushroom anticancer proteins

Mushroom polysaccharides are the most extensively investigated among all the bioactive substances of mushrooms. However, bioactive proteins constitute another important part of functional components in mushrooms, which also attract increasing attention due to their pharmaceutical potential and possibility to apply protein engineering with specified properties. Mushroom proteins with anticancer activity can be divided by two groups: proteins with direct antiproliferative activity on cancer cells and immunomodulating proteins. Lectins form a group which can possess both mechanisms of action. Cancer cell lines Hep G2 and MCF-7 have been used the most frequently for examination of mushroom proteins antiproliferative activity *in vitro* (Table 2).

Table 2. Cytotoxicity of mushroom proteins against tumor cell lines

Protein group	Taxa	IC50 for tumor cell lines, μM		Ref.
		Hep G2	MCF-7	
Lectin	*Pholiota adiposa*	2.1	3.2	[17]
	Hericium erinaceum	56.1	76.5	[18]
	Russula lepida	1.6	0.9	[19]
	Russula delica	0.88	0.52	[20]
Laccase	*Tricholoma mongolicum*	0.65	1.4	[21]
	Clitocybe maxima	12.3	3.0	[22]
	Abortiporus biennis	12.5	6.7	[23]
	Agrocybe cylindracea	5.6	6.5	[24]
	Hericium coralloides	> 60.0		[25]
Ribosome inactivating protein	*Hypsizigus marmoreus*	0.15	5.0	[26]

Lectins are multivalent proteins or glycoproteins of nonimmune origin, which can specifically recognize and reversibly bind to carbohydrate moiety of glycoconjugates without disturbing the covalent structure of any recognized glycoside ligand [27]. Biological role of lectins is very diverse: they participate in the mobilization and transportation of sugars, cell recognition, growth regulation, and differentiation, as well as in the process of the penetration of parasitic fungi into a host organism and in the relation between the fungal organism and a higher plant in the process of mycorrhization [27]. Lectins are probably the most extensively investigated of all the mushroom proteins. It is worth to note that owing to unique capacity of specifically binding to glycoconjugates, mushroom lectins find applications in study of the modifications in membrane glycoconjugates and cancer formation, sorting of mutant and tumor cells, diagnostics, and creation of prodrugs. Apart from cell agglutinating activity, some lectins possess mitogenic activity toward certain cell types, e. g., immune cells, and/or direct antiproliferative activity toward tumor cells [28].

Among lectins with direct cytotoxicity against cancer cells mention may be made of ricin B-like lectin from *Clitocybe nebularis* that can recognize human blood group A determinant carbohydrates and possesses antiproliferative activity specific to human leukemic T cells [28]. Belonging to the same type glycoprotein lectin from fruiting bodies of *G. frondosa* is cytotoxic against HeLa cells [29]. Lectins from *Polyporus adusta* and *Ganoderma carpense* exhibit both mitogenic activity toward spleen cells and antiproliferative activity toward cancer cells [29]. Anti-

proliferative activity of some lectins toward human cell lines Hep G2 and MCF-7 is summarized in the Table 2.

At the same time, some mushroom lectins appertain to a new family of bioactive proteins which is referred to as fungal immunomodulatory proteins. Thus, a fungal immunomodulatory lectin referred to as fve from *Flammulina velutipes* demonstrated potent adjuvant properties enhancing T-helper type 1 antigen-specific and peripheral blood lymphocyte immune responses, thus leading to strong antitumor effect [29]. It also was shown that fve could enhance INF-γ production *via* the p38-mitogen activated protein kinase signaling pathway which is considered being the key factor for antitumor activity of fve *in vivo*.

Another immunomodulatory lectin was isolated from the fruiting bodies of *A. cylindracea* and showed a six-fold increase in mitogenic response when mouse splenocytes were incubated with 2 μM of aforementioned lectin [29]. Bolesatine is a toxic glycoprotein from *Boletus satanas* with hemagglutinating activity which displays biphasic action: mitogenic activity toward human and rat lymphocytes at low doses and inhibiting effect at higher doses. It also elicits the release of IL-1 and IL-2 in supernatants from mononuclear cell cultures [30]. Lectin from *Volvariella volvacea* expresses mitogenic activity toward human peripheral lymphocytes, inhibits generation of bovine serum albumin-induced Arthus reaction, and stimulates transcriptional expression of IL-3, IL-4, IFN-γ, TNF-α, lymphotoxin, and IL 2-receptor [29].

Lin et al. [29] isolated an immunomodulatory protein of non-lectin origin GMI from *Ganoderma microsporum* and investigated its activity in suppressing tumor invasion and metastasis. It was found that GMI, in a dose-dependent manner, inhibits EGF mediated migration and invasion in A549 cells by several pathways such as inhibiting EGF-induced phosphorylation, EGF-induced activation of Cdc42 GTPase, activation of EGF receptor and Ala pathway kinases.

Hemolysin is an agent or a substance that causes the destruction of red blood cells, thereby liberating hemoglobin [31]. Thereby, hemolysin can be distinguished similarly to lectin namely by interaction with red blood cells. Few hemolytic proteins isolated from mushrooms, among them hemolysins from *Pleurotus ostreatus, Pleurotus eryngii, Pleurotus nebrodensis,* and *Amanita phalloides,* exhibited potential anti-proliferative activity on several cancer cell lines *in vitro* [32]. Thus, phallolysin from *A. phalloides* is cytotoxic to HeLa cells and ascites tumor cells (the mouse sarcoma P43), *P. ostreatus* hemolysin possess antiproliferative activities against HT-1080 and MCF-7 cells, *P. eryngii* hemolysin reduces the viability of L1210 cells, finally, nebrodeolysin from *P. nebrodensis* showed strong cytotoxicity against Lu-04, Bre-04, HepG2, L929 and HeLa cells with apoptosis induction in L929 and HeLa cells [32].

Laccases deserve special interest among mushroom enzymes because of their potent role in the biodegradation of lignin and phenolic pollutants. In addition, recently was found that some mushroom laccases possess direct cytotoxicity *in vitro*, particularly toward Hep G2 and MCF-7 cell lines (see Table 2). Laccases (benzenediol: oxygen oxidoreductase) form a class of ligninolytic enzymes which demonstrate a rather low degree of specificity with regard to the reducing substrate: they catalyze the oxidation of *ortho*- and *para*-diphenols and aromatic amines by removing an electron and a proton from a hydroxyl group to generate a free radical [29]. It is likely that cytotoxicity of laccases can be associated with their capacity to oxidize wide range of substrates.

Recently was shown that antiproliferative activity can be attributed to enzymes which inactivate ribosomes by eliminating one or more adenosine residues from rRNA [28]. During the past decade, mushroom ribosome inactivating proteins were isolated from several species including *Calvatia caelata, F. velutipes, H. marmoreus, Lyophyllum shimeiji,* and *Pleurotus tuber-regium* [28]. Thus, calcaelin from *C. caelata* demonstated anti-mitogenic activity toward spleen cells and anti-proliferative activity toward breast cancer cells [29].

Ribonucleases distinct from ubiquitin-like peptides and proteins were isolated from several mushroom species. One of RNase was isolated from *Pleurotus sajor-caju*; it exerts an antiproliferative action on hepatoma and leukemia cells, and anti-mitogenic action on mouse spleen cells [29].

Ubiquitin-conjugated proteins including cell cycle regulatory proteins, p53 tumor suppressor, the transcriptional regulator NF-κB and its inhibitor, numerous transcription factors, and the *mos* protooncogen, are targets for degradation by the 26S proteasome. The ubiquitin-mediated pathway regulates cell-cycle progression, signal transcription regulation, receptor down-regulation, endocytosis, immune response, development, and apoptosis. Defects in ubiquitin-mediated events may be involved in the development of pathological conditions including malignant transformation [29]. Particularly, ubiquitin-like peptide from the mushroom *C. caelata* demonstrates anti-mitogenic activity toward mouse splenocytes and antiproliferative activity toward human breast cancer cells [29]. Similarly, the ubiquitin-like peptide from *A. cylindracea* exerts immunostimulating and antiproliferative activities [29].

Antifungal protein lentin was isolated from fruiting bodies of *L. edodes*. It was shown [33] that lentin exhibited cytotoxicity toward cell line L1210 with IC_{50} 0.2 μM supposedly *via* inhibition of translation.

Cytotoxic terpenoids from mushrooms

Terpenoid is any of a class of hydrocarbons that consist of terpenes attached to an oxygen-containing group [31]. In turn, terpene is any from the class of hydrocarbons consisting of two or more isoprene (C_5H_8) units joined together [31].

Numerous investigations are devoted to cytotoxic activity of basidiomycete *Ganoderma lucidum* triterpenoids (containing 6 isoprene units). More than 150 triterpenoids were isolated from fruiting bodies of the genus *Ganoderma* [34]. Polyoxygenated

ganoderic triterpene acids T, V, W, X, Y, and Z with lanostane skeleton, isolated from *G. lucidum*, exhibited cytotoxic activity *in vitro* toward HTC cell line [35]. It was shown [36] that triterpenoid fraction from the fruiting bodies of *G. lucidum* contains ganoderic acid F and exhibits anticancer and antimetastatic activity through inhibition of angiogenesis which was induced by tumor. Triterpene enriched fraction from *G. lucidum* mycelium inhibited the growth of Huh-7 cells with IC_{50} about 100 μg/ml, though showed low toxicity (IC_{50} > 1 mg/ml) for normal hepatocytes [37]. This fraction inhibited protein kinase C, but activated other protein kinases (which is usually occurs during mitosis) and arrested cell cycle in G2 phase in the hepatoma cells, however it didn't have such effect in normal hepatocytes [37]. Previously it was shown that the mechanism of this fraction cytotoxicity for tumor cells includes oxidative stress [38]. Three triterpene aldehydes with lanostane skeleton were derivated from fruiting bodies of *G. lucidum* and named lucialdehydes A, B, and C [39]. Lucialdehydes B and C exhibited cytotoxicity toward LLC, T-47D, Sarcoma 180 and Sarcoma Meth-A cell lines. Lucialdehyde C revealed the highest cytotoxicity toward tested cells with ED_{50} 10.7, 4.7, 7.1, and 3.8 mg/l, respectively. It was elucidated [40], that intracellular triterpenoids from *G. lucidum* can be produced predominantly on the latter stages of submerged fermentation, have different composition and cytotoxicity toward K562 cells.

Mushroom terpenoids can be cytotoxic not only for cancer cells, but also for normal cells; therefore creation of semisynthetic analogues with specified properties is a promising approach. Thus, in 1963 as a result of screening from about 600 mushrooms it was determined [41] that *Lampteromyces japonicus* has a high anticancer activity associated with toxic substance lamterol. Cytotoxic tricyclic sesquiterpene (containing 3 isoprene units) illudin S (lamterol) can be also synthesized by *Omphalotus olearius* [4]. It is believed that illudin S undergoes activation by glutathione. The activated form is then capable of covalently bind to DNA which halts DNA replication [4] and leads to cell death in interphase G1-S [42]. Illudin S itself is too toxic to be used as a clinical drug. Consequently, a semisynthetic illudin analog HMAF, that demonstrated a superior therapeutic profile and lower toxicity, employed in clinical trials. *In vitro* investigations of HMAF have shown that IC_{50} for cancer cell lines ranging from 160 nM in sensitive MCF-7 to 17 μM in relatively insensitive B16 cells [43]. *In vivo* antitumor activity was consistent with *in vitro* sensitivity. HMAF was very effective in human tumor xenograft models, including MX-1, MV522, and HT-29, but not B16 or P388 [43]. Thus, complete regression recorded in 29 of 30 animals bearing MX-1 tumors after administration of HMAF intravenously at the doses of 3–7.5 mg/kg daily for 5 days. Extensive tumor shrinkage was also observed with MV522, and significant tumor growth inhibition was obtained with HT-29 when animals received intraperitoneal injections at the doses ranging from 3.75 to 7.5 mg/kg during 5 days [43]. Complete regressions were also observed in individual animals with MV522 and HT-29. HMAF passed successfully clinical trial phase I [44], though during the phase II the lack of efficiency was revealed [45, 46], and subsequent studies have stopped.

Anticancer activity of mushroom steroids, phenols, and dietary fiber

Among mushroom steroids the glycosylated form of ergosterol peroxide from the methanol extract of *Cordyceps sinensis* was found [47] to be an inhibitor of proliferation of K562, Jurkat, WM-1341, HL-60, and RPMI-8226 tumor cell lines.

Anticancer sterol from *Sarcodon aspratus* selectively suppressed the growth of HT29 cancer cells, but not WI38 normal human fibroblasts [48]. Investigations of anticancer mechanism have shown that aforementioned sterol induces expression of the cyclin-dependent kinase inhibitor 1A, thus causing cell cycle arrest and apoptosis in HT29 cells.

Phenols can possess anticancer activity as preventive agent with antioxidant properties and also can exhibit direct cytotoxicity toward cancer cells. Thus, hericenones A and B from *Hericium erinaceum* demonstrated complete growth inhibition of HeLa cells at the concentrations of 100 μg/ml and 6.3 μg/ml respectively [49]. It was suggested, that potent cytotoxicity of hericenone B may be due to γ-lactam and its N-substituent.

Mushroom cell walls contain high molecular weight materials which cannot be digested or absorbed by human intestine, but absorb carcinogenic substances (heavy metals, free radicals, etc.): chitin, homo- and heteropolysaccharides [4].

Quantitative and qualitative composition of fungal mycelia depends on various cultivation conditions, especially on the substrate for cultivation. Synthetic easily accessible media can suppress generation of some valuable secondary metabolites [50]. In this aspect the selection of the cultivation media is one of the important stages for obtaining of biologically active substances of fungal mycelia. The most preferred can be considered natural substrates — wastes of agriculture [51].

ANTICANCER EXTRACTS FROM MUSHROOMS

The above substances having antitumor activity were extracted from the fungi with various solvents. Ethanol, methanol, ethyl acetate, and hot water extracts of fruiting bodies and mycelia are often used in preclinical studies of mushroom antitumor activity *in vitro* and *in vivo*. Occasionally anticancer activity of other extracts (acetone, ether, chloroform, cold water, etc.) has been tested.

The first demonstration of mushroom extract antitumor activity has been conducted in 1957 by Lucas et al. [52] using extracts from the fruiting bodies of *Boletus edulis* and other holobasidiomycetes against Sarcoma-180.

Ethanol extracts

Among mushroom extracts an ethanol extract probably finds the most extensive application. Thus, ethanol extracts of *Pleurotus florida* and *Calocybe indica* caused apoptosis in T24 cell line in the dose-dependent manner [53].

The effects of ethanol extracts from *G. frondosa, G. lucidum, Hericium erinaceus,* and *L. edodes* fruiting bodies, spores, and cultured broth were assessed for modulation of cell proliferation and apoptosis in CH72 cancer cells and C50 normal cells [54]. Among above-listed ethanol extracts only *L. edodes* extract significantly decreased CH72 cell proliferation; at the same time, none of the extracts changed proliferative response of normal C50 cells. Cell cycle analysis demonstrated that *L. edodes* extract induced a transient G1 phase arrest in CH72 cells.

It was demonstrated [55], that *A. brasiliensis* 50% ethanol extract in concentration 0.9 mg/ml and hot water extract in concentration 0.7 mg/ml caused morphological changes and significantly reduced CAL27 cell viability after 48-h treatment. Both extracts induced apoptotic cell death in CAL27 *via* the release of cytochrome c from mitochondria into the cytoplasm and activation of caspase-3 *in vitro*.

Comparison of the effects of extracellular, intracellular polysaccharides, and ethanol extracts of *Tremella mesenterica* revealed [56] that only ethanol extract induced apoptosis in A549 cells. Such effect was provoked by activating a mitochondrial pathway: disruption of mitochondrial transmembrane potential, the production of reactive oxygen species, and the activation of caspase-3 protein in ethanol extract-treated A549 cells.

Methanol extracts

The methanol extracts of *Pleurotus ostreatus* and *Pleurotus salmoneostramineus* showed suppressive effect against growth of HT-29 cell line with survival rates of 39.9 and 40.7% at the concentration 500 μg/ml, while survival rate was more than 50% when *Pleurotus cornucopiae* methanol extract was employed [57].

Anticancer activity can be not only species-, but also a strain-dependent characteristic. This point of view was illustrated by another study dedicated to comparison of antiproliferative activity of methanol extracts from European and Asian *G. lucidum* [58]. The research revealed that methanol extract of Chinese strain as opposite to French strains didn't inhibit the growth of HT29 cell line. However, all investigated extracts exhibited different cytotoxic activity (with IC_{50} varied from 68 to 171 μg/ml) toward PC-3 and MCF7 cell lines. It was established [58] that methanol extracts of *G. lucidum* contain triterpenoids which apparently can cause apoptosis of cancer cells.

Ethyl acetate extracts

One of the studies [59] was associated with antiproliferative activity of ethyl acetate and culture broth extracts of *Coprinus comatus*. The investigation showed that IC_{50} value for MCF7 cells was 76 μg/ml for culture broth extract and 32 μg/ml for ethyl acetate extract. The results also revealed that both extracts significantly affected IκBα phosphorylation in a dose-dependent manner. In addition, the data obtained showed that only ethyl acetate extracts inhibited the activity of IκB kinase enzyme complex at close to 90% as compared to the control of the untreated sample.

Ethyl acetate extract from *Antrodia camphorata* fruiting bodies decreased the cell growth of Hep G2 and PLC/PRF/5 cells in a dose dependent manner [60]. In Fas/APO-1 positive-Hep G2 cells, extract increased the expression level of Fas/APO-1 and its two forms of ligands, membrane-bound Fas ligand and soluble Fas ligand, in a p53-indenpendent manner. In addition, extract also initiated mitochondrial apoptotic pathway through regulation of Bcl-2 family proteins expression, release of cytochrome c, and activation of caspase-9 both in Hep G2 and PLC/PRF/5 cells. Furthermore, it also inhibited the cell survival signaling by enhancing the amount of IκBα in cytoplasm and reducing the level and activity of NF-κB in the nucleus, and subsequently attenuated the expression of Bcl-X(L) in Hep G2 and PLC/PRF/5 cells.

Hot water extracts

Oral administration of mixture (known as M8) from hot water extracts of the 7 medicinal mushrooms (*Armillaria mellea, G. frondosa, Garnoderma frondosa, Cordyceps militaris, Hericium erinaceus, T. versicolor,* and *Agaricus blazei)* and the medicinal plant *Lycium barbarum* resulted in a dose-dependent tendency to inhibit lung metastasis after intravenous injection of colon26-L5 cells [61]. Treatment with M8 resulted in the increase of T cell and B cell mitogenic stimuli as well as in increased production of IFN-γ and IL-4 by splenocytes.

Hot water extract of *Inonotus obliquus* exerts inhibitory activity against the proliferation of HT-29 in a dose-dependent manner *via* the induction of apoptosis and inhibition of the cancer growth through up-regulation of the expression of proapoptotic proteins (bcl-2-like protein 4 and caspase-3) and down-regulation of antiapoptotic protein Bcl-2 [62].

Other extracts

Acetone extracts of seven edible mushroom fruiting bodies (*Hericium erinaceum, Lentinus lepides, Leucopaxillus giganteus, Lyophillum decastes, Pleurocybella porrigens, Pleurotus cornucopiae,* and *Sarcodon aspratus*) were tested against HL60 cell line [63]. Antiproliferative activity of the *S. asparatus* extract was the most prominent. The active substance ergosterol peroxide completely inhibited growth and induced apoptosis of HL60 cells at a concentration 10.7 μg/ml.

Ether and ethanol extracts of *Piptoporus betulinus* mycelia highly decreased the viability of LS180 cancer cells, slightly inhibiting proliferation and tumor cell adhesion in a time- and dose-dependent manner [64]. At the same time, the cytotoxicity of the extracts against CCD 841 CoTr normal cells was observed only at the highest studied concentration.

Ethanol, ethyl acetate, chloroform, and, in some cases, culture liquid extraction of 12 medicinal mushrooms were screened for their effect on the viability of HPAF-II and PL45 cell lines with various treatment doses (50–500 μg/ml) [65]. The best results were obtained for *Cyathus striatus* culture luquid extract, even at lower concentrations (1–50 μg/ml) it showed a significant decrease in cell viability.

To the best of our knowledge, there was no positive evidence of anticancer activity attributed to cold water extracts of medicinal mushrooms. Thus, cold water extract of *Agaricus brasiliensis* didn't influence intestinal immunoglobulin A level or TNF-α, IFN-γ, and IL-10 levels in serum of experimentally immunodepressed mice with cyclophosphamide [66]. Though, cold water extracts of medicinal mushrooms can cause other effects, such as hepatoprotective [67].

NOVEL APPROACHES OF CANCER TREATMENT USING MUSHROOM PRODUCTS

Vaccinotherapy

Vaccine preparations with preventive properties against liver and cervical cancer associated with hepatitis B and human papillomatosis infections were developed in Belgium and USA [68]. At the same time, to date there is no vaccine on commercial scale aimed to cure existing tumors, metastases or relapses. Addition of immunomodulating substances of natural and synthetic origin to vaccines can sufficiently enhance their anticancer properties.

Thus, there were substantiated doses and schemes of *L. edodes* polysaccharide fraction administration along and design of it combination with vaccine on the basis of autological glycopeptides of Ehrlich's carcinoma, Sarcoma 37, LLC, L1210, and B16 cell lines [68]. Such preparations enhance cytolytic activity of lymphocytes, metabolic activity of peritoneal macrophages, and cytolytic activity of blood serum in the presence of complement in intact animals and in Sarcoma 37 bearing animals.

An immunomodulating protein Ling Zhi-8 from mycelia of *G. lucidum* with stimulatory activity on dendritic cells was recently identified [69]. The large-scale amplification of recombinant protein Ling Zhi-8 has been achieved in a patented yeast system. It was shown [69] that aforementioned protein significantly increases the efficacy of a cancer DNA vaccine in a preclinical tumor model.

Nanovectors for drug delivery

Nowadays preparation of nanoparticles using "green" chemistry and bioprocess approach is advantageous over physical and chemical methods owing to its environmental significance. In this aspect, living organisms are highly potential for the production of nanoparticles. Irregular shaped gold nanoparticles were synthesized by photo-irradiation technique using *Pleurotus florida* as a reductant [70]. It was suggested that flavins (flavo proteins) present in the mushroom extract are responsible for the reduction of ions into nanoparticles: when exposed to sunlight flavins absorb photons of energy and can act in reduction-oxidation reactions. Obtained nanoparticles showed cytotoxicity against A-549, K-562, HeLa, and MDA-MB cancer cells and no effect against Vero normal cells.

CONCLUDING REMARKS

Analysis of literature data has shown that anticancer activity of mushroom products provoked increasing interest of researchers. The most abundant amount of investigations among anticancer substances of mushroom origin are attached to polysaccharides, polysaccharide-protein complexes, lectins and terpenoids. Only a few of substances has passed initial phases of clinical trials: D- and MD-fractions of *G. frondosa,* PSP and PSK from *T. versicolor,* lentinan from *L. edodes,* schizophyllan from *S. commune,* and HMAF from *L. japonicus.*

Mushroom lectins are a group of proteins which can possess as immunomodulating as well as direct cytotoxic activity toward cancer cells. It is also possible to use specificity of lectins to glucoconjugate in order to study the modifications in membrane glucoconjugates and cancer formation, sorting of mutant and tumor cells, diagnostics, and creation of prodrugs. There are some rarely mentioned anticancer mushroom proteins as hemolysin proteins, enzyme laccase, ribosome inactivating proteins, and ubiquitin-conjugated proteins which display direct cytotoxic activity *in vitro.* Proteins can be a convenient tool for medical treatment because of the possibility of genetic engineering and large-scale amplification.

Anticancer activity of ethanol, methanol, ethyl acetate, and hot water extracts of fruiting bodies and mycelia from mushrooms of different ecological and systematical groups was established. Some examples of mushroom extracts investigations are provided.

Substances contained in mushrooms may be able to interfere with tumor through a variety of mechanisms, e.g., by enhancing the host's antioxidant capacity or by absorption of carcinogens. Yet other mushroom constituents may inhibit promotion or progression of cancer by exerting direct cytotoxic effects on tumor cells, by interfering with tumor angiogenesis, or by upregulating immune and/or non-immune tumor-suppressive mechanisms. When combined with traditional anticancer treatment, mushroom products were shown to lower side effects.

Vaccinotherapy is a new direction in cancer treatment and prevention. In this aspect mushroom immunomodulating adjuvants can be an efficient component of traditional and DNA vaccines.

Creation of nanovectors for drug delivery and diagnostic is also a new approach in cancer therapy. Mushroom mycelia can be an ecologically clean and efficient reductant in conversion of ions to nanoparticles with selective cytotoxicity toward cancer cells.

REFERENCES

1. Ferrari M. Cancer nanotechnology: opportunities and challenges. Nature 2005; **5**: 161–71.

2. Huennekens FM. Tumor targeting: activation of prodrugs by enzyme-monoclonal antibody conjugated. Trends Biotech 1994; **12**: 234–9.

3. Hunt KK, Vorburger SA. Hurdles and hopes for cancer treatment. Science 2002; **297**: 415–6.

4. Wasser SP, Weis AL. Medicinal properties of substances occurring in higher Basidiomycetes mushrooms: current perspectives (review). Int J Med Mushr 1999; **1**: 31–62.

5. Wasser SP. Modern View on Current Status, Future Trends, and Unsolved Problems in Studies of Medicinal Mushrooms. In: J Zhang, H Wang, M Chen, eds. Proceedings of the 18th Congress of the International Society for Mushroom Science. Beijing, 2012: 401–15.

6. Dai Yu-C, Yang Z-L, Cui B-K, ***et al.*** Species diversity and utilization of medicinal mushrooms and fungi in China (review). Int J Med Mush 2009; **11**: 287–302.

7. Zong A, Cao H, Wang F. Anticancer polysaccharides from natural resources: a review of recent research. Carbohydr Polym 2012; **90**: 1395–410.

8. Smith JE, Zong A, Rowan NJ, ***et al.*** Medicinal Mushrooms: their therapeutic properties and current medical usage with special emphasis on cancer treatments. London: Cancer Research UK, 2002. 260 p.

9. Chihara G, Maeda YY, Hamuro J. Inhibition of mouse sarcoma 180 by polysaccharides from *Lentinus edodes* (Berk.) Sing. Nature 1969; **222**: 687–8.

10. Taguchi T. Clinical efficacy of lentinan on patients with stomach cancer: end point results of a four year follow-up survey. Cancer Detect Prev 1987; **1**: 333–49.

11. Chihara G. Medical Aspects of Lentinan Isolated from *Lentinus edodes* (Berk.) Sing. In: ShT Chang, JA Buswell, SW Chiu, eds. Proceedings of the First International Conference on Mushroom Biology and Mushroom Products. Hong Kong, 1993: 261–6.

12. Ooi VEC, Liu F. Immunomodulation and anti-cancer activity of polysaccharide-protein complexes. Curr Med Chem 2000; **7**: 715–29.

13. Klechak IR, Antonenko LO. The biotechnology on the basis of the higher Basidiomycetous mushrooms of genus *Coriolus* Quel. Res Bull NTUU "KPI" 2012; **83**: 41–9 (in Ukrainian).

14. Maehara Y, Tsujitani S, Saeki H. Biological mechanism and clinical effect of protein-bound polysaccharide K (KRESTIN): review of development and future perspectives. Surg Today 2012; **42**: 8–28.

15. Yang QY, Hu YJ, Li XY, ***et al.*** A New Biological Response Modifier — PSP. In: ShT Chang, JA Buswell, SW Chiu, eds. Proceedings of the First International Conference on Mushroom Biology and Mushroom Products. Hong Kong, 1993: 247–59.

16. Zong A, Cao H, Wang F. Anticancer polysaccharides from natural resources: a review of recent research. Carbohydr Polymers 2012; **90**: 1395–410.

17. Zhang GQ, Sun J, Wang HX, ***et al.*** A novel lectin with antiproliferative activity from the medicinal mushroom *Pholiota adiposa*. Acta Biochimica Polonica 2009; **56**: 415–21.

18. Li Y, Zhang G, Ng TB, ***et al.*** A novel lectin with antiproliferative and HIV-1 reverse transcriptase inhibitory activities from dried fruiting bodies of the monkey head mushroom *Hericium erinaceum*. J Biomed Biotech 2010. doi: 10.1155/2010/716515.

19. Zhang G, Sun J, Wang H, ***et al.*** First isolation and characterization of a novel lectin with potent antitumor activity from a Russula mushroom. Phytomedicine 2010; **17**: 775–81.

20. Zhao S, Zhao YC, Li SH, ***et al.*** A novel lectin with highly potent antiproliferative and HIV-1 reverse transcriptase inhibitory activities from the edible wild mushroom *Russula delica*. Glycoconj J 2010; **27**: 259–65.

21. Li M, Zhang G, Wang H, ***et al.*** Purification and characterization of a laccase from the edible wild mushroom *Tricholoma mongolicum*. J Microbiol Biotech 2010; **20**: 1069–76.

22. Zhang G, Sun J, Wang H, ***et al.*** First isolation and characterization of a novel laccase from the edible mushroom *Clitocybe maxima*. Process Biochem 2010; **45**: 627–33.

23. Zhang GQ, Tian T, Liu YP, ***et al.*** A laccase with antiproliferative activity against tumor cells from a white root fungus *Abortiporus biennis*. Process Biochem 2011; **46**: 2336–40.

24. Xu W, Huang JJ, Cheung PC. Extract of *Pleurotus pulmonarius* suppresses liver cancer development and progression through inhibition of VEGF-induced PI3K/AKT signaling pathway. PLOS One 2012; **7**: e34406. doi:10.1371/journal.pone.0034406.

25. Zou YJ, Wang HX, Ng TB, ***et al.*** Purification and characterization of a novel laccase from the edible mushroom *Hericium coralloides*. J Microbiol 2012; **50**: 72–8.

26. Wong JH, Wang HX, Ng TB. Marmorin, a new ribosome inactivating protein with antiproliferative and HIV-1 reverse transcriptase inhibitory activities from the mushroom *Hypsizigus mamroreus*. Appl Microbiol Biotechnol 2008; **81**: 669–74.

27. Mikiashvili N, Elisashvili V, Worku M. Purification and characterization of a lectin isolated from the submerged cultivated mycelium of grey polypore *Cerrena unicolor* (Bull.) Murrill (*Aphyllophoromycetideae*). Int J Med Mushr 2009; **11**: 61–8.

28. Xu X, Yan H, Chen J, ***et al.*** Bioactive proteins from mushrooms. Biotech Adv 2011; **29**: 667–74.

29. Ng TB. Peptides and proteins from fungi. Peptides 2004; **25**: 1055–73.

30. Ennamany R, Kretz O, Badoc A, ***et al.*** Effect of bolesatine, a glycoprotein from *Boletus satanas,* on rat thymus *in vivo*. Toxicol 1994; **89**: 113–8.

31. The American Heritage Science Dictionary. Published by Houghton Mifflin, 2002. 704 p.

32. Lv H, Kong Y, Yao Q, ***et al.*** Nebrodeolysin, a novel hemolytic protein from mushroom *Pleurotus nebrodensis* with apoptosis-inducing and anti-HIV-1 effects. Phytomedicine 2009; **16**: 198–205.

33. Ngai PHK, Ng TB. Lentin, a novel and potent antifungal protein from shitake mushroom with inhibitory effects on activity of human immunodeficiency virus-1 reverse transcriptase and proliferation of leukemia cells. Life Sciences 2003; **73**: 3363–74.

34. Boh B, Berovic M, Zhang J, ***et al.*** *Ganoderma lucidum* and its pharmaceutically active compounds. Biotech Ann Rev 2007; **13**: 265–301.

35. Toth JO, Luu B, Ourisson G. Les acides ganoderiques T à Z : triterpenes cytotoxiques de Ganoderma lucidum (Polyporacée). Tetrahedron Lett 1983; **24**: 1081–84.

36. Kimura Y, Taniguchi M, Baba K. Antitumor and antimetastatic effects on liver of triterpenoid fractions of *Ganoderma lucidum:* mechanism of action and isolation of an active substance. Anticancer Res 2002; **22**: 3309–18.

37. Lin Sh-B, Li Ch-H, Lee Sh-Sh, ***et al.*** Triterpene-enriched extracts from *Ganoderma lucidum* inhibit growth of hepatoma cells via suppressing protein kinase C, activating mitogen-activated protein kinases and G2-phase cell cycle arrest. Life Sciences 2003; **72**: 2381–90.

38. Lin Sh-B, Li Ch-H, Chen YR, ***et al.*** Triterpene extract from *Ganoderma lucidum* inhibits growth of hepatoma Huh7 cells: involvement of oxidative stress induction. In: ZB Lin, ed. Ganoderma: Genetics, Chemistry,

Pharmacology and Therapeutics. Beijing: Beijing Medical University Press, 2002: 176–82.

39. Gao JJ, Min BS, Ahn EM, ***et al.*** New triterpene aldehydes, lucialdehydes A-C, from *Ganoderma lucidum* and their cytotoxicity against murine and human tumor cells. Chem Pharmacol Bull 2002; **50**: 837–40.

40. Yu SP, Zhang JS, Tang QJ, ***et al.*** Correlation between intracellular triterpenes from mycelia of *Ganoderma lucidum* in different growth stages and inhibition effect on tumor cells. Mycosystema 2004; **23**: 548–54.

41. Nakanishi K, Tada M, Yamada Y, ***et al.*** Isolation of lampterol, an antitumour substance from *Lampteromyces japonicus*. Nature 1963; **197**: 292.

42. Kelner MJ, Mc Morris TC, Beck WT, ***et al.*** Preclinical evaluation of illudins as anticancer agents. Cancer Res 1987; **47**: 3186–9.

43. MacDonald JR, Muscoplat ChC, Dexter DL, ***et al.*** Preclinical antitumor activity of 6-hydroxymethylacylfulvene, a semisynthetic derivative of the mushroom toxin illudin S. Cancer Res 1997; **57**: 279–83.

44. Eckhardt SG, Baker ShD, Britten CD, ***et al.*** Phase I and pharmacokinetic study of irofulven, a novel mushroom-derived cytotoxin, administered for five consecutive days every four weeks in patients With advanced solid malignancies. J Clinical Oncol 2000; **18**: 4086–97.

45. Dowell JE, Johndon DH, Rogers JS, ***et al.*** A phase II trial of 6-hydroxymethylacylfulvene (MGI-114, irofulven) in patients with advanced non-small cell cancer previously treated with chemotherapy. Invest New Drugs 2001; **19**: 85–8.

46. Sherman CA, Herndon JE, Watson DM, ***et al.*** A phase II trial of 6-hydroxymethylacylfulvene (MGI-114, irofulven) in patients with relapsed or refractory non-small cell lung cancer. Lung Cancer 2004; **45**: 387–92.

47. Bok JW, Lermer L, Chilton J, ***et al.*** Antitumor sterols from the mycelia of *Cordyceps sinensis*. Phytochem 1999; **51**: 891–8.

48. Kobori M, Yoshida M, Ohnishi-Kameyama M, ***et al.*** 5alpha,8alpha-epidioxy-22E-ergosta-6,9(11),22-trien-3beta-ol from an edible mushroom suppresses growth of HL60 leukemia and HT29 colon adenocarcinoma cells. Biol and Pharm Bull 2006; **29**: 755–9.

49. Kawagishi H, Ando M, Mizuno T. Hericenone A and B as cytotoxic principles from the mushroom *Hericium erinaceum*. Tetrahedron Lett 1990; **31**: 373–6.

50. Peng X, Zhong-Yang D, Zhu Q, ***et al***. Improved production of mycelial biomass and ganoderic acid by submerged culture of *Ganoderma lucidum* SB97 using complex media. Enzyme Microb Technol 2008; **42**: 325–31.

51. Ivanova TS, Bisko NA, Krupodorova TA, Barshteyn VYu. Breadcrumb as a New Substrate for *Trametes versicolor* and *Schizophyllum commune* Submerged Cultivation. Korean J Microbiol Biotechnol 2014; **42**: 67–72.

52. Lucas EH, Montesono R, Pepper MS, ***et al.*** Tumor inhibitors in *Boletus edulis* and other holobasidiomycetes. Antibiot Chemother 1957; **7**: 1–4.

53. Selvi S, Umadevi P, Murugan S, ***et al.*** Anticancer potential evoked by *Pleurotus florida* and *Calocybe indica* using T24 urinary bladder cancer cell line. Int African J Biotech 2011; **10**: 7279–85.

54. Gu Y-H, Belury MA. Selective induction of apoptosis in murine skin carcinoma cells (CH72) by an ethanol extract of *Lentinula edodes*. Cancer Lett 2005; **220**: 21–8.

55. Fan MJ, Lin YC, Shih HD, ***et al.*** Crude extracts of *Agaricus brasiliensis* induce apoptosis in human oral cancer CAL 27 cells through a mitochondria-dependent pathway. In Vivo 2011; **25**: 355–66.

56. Chen N-Y, Lai H-H, Hsu T-H, ***et al.*** Induction of apoptosis in human lung carcinoma A549 epithelial cells with an ethanol extract of *Tremella mesenterica*. Bioscience Biotech Biochem 2008; **72**: 1283–9.

57. Kim J-H, Kim S-J, Park H-R, ***et al.*** The different antioxidant and anticancer activities depending on the color of oyster mushrooms. J Med Plants Res 2009; **3**: 1016–20.

58. Welti S, Moreau P-A, Azaroual N, ***et al.*** Antiproliferative activities of methanolic extracts from a neotropical *Ganoderma* species *(Aphyllophoromycetideae)*: identification and characterization of a novel ganoderic acid. Int J Med Mushr 2010; **12**: 17–31.

59. Asatiani MD, Wasser SP, Nevo E, ***et al.*** The shaggy inc cap medicinal mushroom, *Coprinus comatus* (O.F.Mull.:Fr.) Pers. (*Agaricomycetideae*) substances interfere with H_2O_2 induction of the NF-ϰB pathway through inhibition of IϰBα Phosphorylation in MCF7 breast cancer cells. Int J Med Mushr 2011; **13**: 19–25.

60. Hsu YL, Kuo YC, Kuo PL, ***et al.*** Apoptotic effects of extract from *Antrodia camphorata* fruiting bodies in human hepatocellular carcinoma cell lines. Cancer Lett 2005; **221**: 77–89.

61. Han S-SR, Cho Ch-K, Lee Y-W, ***et al.*** Antimetastatic and immunomodulating effect of water extracts from various mushrooms. J Acupunct Meridian Stud 2009; **2**: 218–27.

62. Lee SH, Hwang HS, Yun JW. Antitumor activity of water extract of a mushroom, *Inonotus obliquus*, against HT-29 human colon cancer cells. Phytother Res 2009; **23**: 1784–9.

63. Takei T, Yoshida MB, Ohnishi-Kameyama M, ***et al.*** Ergosterol peroxide, an apoptosis-inducing component isolated from *Sarcodon aspratus* (Berk.) S. Ito. Biotech Biochem 2005; **69**: 212–5.

64. Cyranka M, Graz M, Kaczor J, ***et al.*** Investigation of antiproliferative effect of ether and ethanol extracts of birch polypore medicinal mushroom, *Piptoporus betulinus* (Bull.:Fr.) P. Karst. (higher *Basidiomycetes*) *in vitro* grown mycelium. Int J Med Mushr 2011; **13**: 525–33.

65. Sharvit LE, Wasser SP, Fares F. The effect of culture liquid ethyl acetate mycelium extracts of medicinal mushrooms on the viability of human pancreatic cancer cells. Int J Med Mushr 2012; **14**: 169–79.

66. Fantuzzi E, Anastacio LR, Nicoli JR, ***et al.*** Aqueous extract of culinary-medicinal royal sun mushroom, *Agaricus brasiliensis* S. Wasser et al. (Agaricomycetideae) effects on immunodepression in mice. Int J Med Mushr 2010; **12**: 227–34.

67. Barbisan LF, Myamoto M, Scolastici C, ***et al.*** Influence of aqueous extract of *Agaricus blazei* on rat liver toxicity induced by different doses of diethylnitrosamine. J Ethnopharmacol 2002; **83**: 25–32.

68. Artamonova GB. Evaluation of the effectiveness of antitumor polysaccharide fractions from the fruit body of the fungus *Lentinus edodes* and glycopeptide vaccine in various experimental tumor models. PhD thesis in biology, speciality 14.01.07 — oncology. RE Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology NAS of Ukraine: Kyiv, 2011. 18 p. (in Ukrainian).

69. Chu CL, Chen DC, Lin CC. A novel adjuvant Ling Zhi-8 for cancer DNA vaccines. Hum Vacc 2011; **7**: 1161–4.

70. Bhat R, Sharanabasava VG, Deshpande R, ***et al.*** Photo-bio-synthesis of irregular shaped functionalized gold nanoparticles using edible mushroom *Pleurotus florida* and its anticancer evaluation. J Photochem Photobiol B 2013; **125**: 63–9.