http://dx.doi.org/10.35630/2199-885X/2021/11/2/9

PHARMACOKINETICS AND PHARMACODYNAMICS OF CHAGA BIRCH MUSHROOM COMPONENTS (INONOTUS OBLIQUUS)

Received 11 May 2021;
Received in revised form 28 May 2021;
Accepted 1 June 2021

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ABSTRACT — The study presents an analysis of the available scientific literature and outcomes of clinical trials on biologically active components of the birch Chaga mushroom *Inonotus obliquus* (*I. obliquus*). Our experimental and clinical studies were aimed at testing a novel plant-based substance for patients with cancer or tumors of various locations. The paper investigates pathogenetic mechanisms and effectiveness of chaga mushroom extracts depending on the topography of the tumor, the stage of the disease and the age of patients, as well as the questions of pharmacokinetics and pharmacodynamics of the components of *I. obliquus*.
As a result of the analysis of the obtained data, the pathogenetically justified clinical application of extracts of the birch chaga mushroom *I. obliquus* in patients with oncological pathology was conceptually presented.
The study allowed us to reveal possible mechanisms of pathogenetic effects of the birch chaga mushroom (*Inonotus obliquus*) extracts obtained by different extraction methods. Analysis of the available data enabled us to deepen our understanding of the effectiveness and mechanisms of the effect of the *I. obliquus* extracts on tumors of various localization. Finally we present recommendations on predicting the possibility of using the *I. obliquus* to improve outcomes in patients with cancer of various etiology and locations.

KEYWORDS — chaga mushroom, Inonotus obliquus, inotodiol, glycans, neuroprotector, hyperglycemia, antioxidants, stroke, apoptosis, proliferation, regeneration, malignancy, cancer.

RELEVANCE

At the current stage of development of medicine it is of particular importance to search for substances that can be used in prevention and supporting measures during treatment of cancer and neurodegenerative diseases. In contrast to strong synthetic drugs with numerous negative side effects which sometimes exceed their benefit, medicinal plants can be assumed to be effective and safe. The analysis by Yen-Nien Hou, Gary Deng, Jun J. Mao (2020) of the data from the Integrative Medicine Service at Memorial Sloan Kettering Nassau cancer center (USA) allowed us to establish that chaga mushroom is in the first place out of the 10 most popular herbs/supplements in 2018 [11, 33]. The chaga mushroom, *Inonotus obliquus* (*I. obliquus*), has been a well-known traditional medicine remedy since the time of Avicenna [1, 3]. However, the systematic pharmacological study of the active substances of *I. obliquus* was started only in the middle of the 20th century, when fundamental medicine claimed the medicinal advantages of the fungus *Inonotus obliquus* in experimental studies with the suppression of cancer cell growth [26]. The chemical composition and spectrum of the therapeutic use of *Inonotus obliquus* as raw material, as well as methods for assessing the resource potential of this fungus, are presented in numerous studies despite the background of insufficient data on the molecular mechanisms of action of mushroom extracts [17]. At the present stage, the medicinal properties of *I. obliquus* have been studied only by 10% [28]. At the same time, the available scientific literature on this issue considers to a greater extent the results of experiments performed on mice, mongrel and genetically modified, or on Danio fish, which is a key factor for choosing the direction of research in the study and systematization of data on the mechanisms of their action on the human body.

Over the past fifty years, ideas about the use of chaga extracts as additives to essential medicines are based mainly on the results of preclinical studies, with a small number of adequately planned experiments and a limited number of studies conducted on cancer patients with cancer of various topography and etiology [4]. Therefore, despite numerous experimental and biochemical studies, the available data on the biological effects of individual components at the present stage does not allow us to give an exhaustive pathogenetic justification for the use of popular additives from chaga mushroom, registered and obtained by various methods [24]. There is also insufficient information to fully decipher the mechanisms of action, substantiate the effectiveness of drugs from the chaga mushroom, prove their advantages and minimize the risks

of side effects. This analysis of available data is also not presented in the archive database of the Integrative Medicine Service at Memorial Sloan Kettering cancer center, which provides a summary of research results indicating the intended uses, side effects, and interactions of herbal medicines for approximately 284 dietary supplements [10].

The known antitumor effect of glycans and glucans that are part of *I. obliquus* is mainly associated with the induction of increased immune activity, and their effect in the composition of chaga extract on malignant tissue has not been sufficiently studied, there are only a few experiments in Vitro confirming their inhibitory effect on the proliferation of cancer cells [5].

Critical unsolved problems in the use of chaga mushroom (*I. obliquus*) extracts and the lack of systematic analysis of available materials on this issue require scientific research to be directed on these matters.

The aim of the study was to generalize data on the medicinal properties of the birch chaga mushroom (*I. obliquus*) and conduct a comparative analysis of the results of its usage in tumors of various locations and etiology.

In this regard, the following tasks were solved:

1. To provide an analysis of available literature data about the current state of the studied issue.
2. To obtain a comparative description of the results of treatment of cancer patients with tumors of different localization and etiology.
3. To determine the possible mechanisms of pathogenetic effects of extracts of the birch chaga mushroom (*Inonotus obliquus*) obtained by different extraction methods based on the available data.
4. To evaluate the effectiveness of the extracts of *Inonotus obliquus* on tumors of various localization.
5. Conceptually substantiate the clinical use of *Inonotus obliquus* extracts in patients with cancer.

MATERIALS AND METHODS

We reviewed available literature data on clinical use of extracts of the *Inonotus obliquus* for the last 20 years, as well as available archival and scientific materials on the results of treatment of patients with cancer of various etiologies and topography of maligning structures.

RESEARCH RESULTS

In contrast to the studies by Draggendorf G.L. (1864) [7], which did not discover useful substances in the birch chaga mushroom (*Inonotus obliquus*) and the presence of alkaloids and glycosides in its content, many researchers found more than 10 amino acids, 40% of which are glutamic acid, tyrosine, serine, threonine, leucine, methionine, histidine and lysine, as well as polysaccharides, in addition to numerous other chemical elements [6, 19]. Previously, data on the health properties of fungal polysaccharides were obtained by analyzing the chemical composition of 651 species and 7 infraspecific taxa from 182 genera of higher homo- and heterobasidiomycetes. It was found that, despite the different chemical composition, the majority of fungal polysaccharides belong to the group of beta-glucans, they have beta (1-->3) bonds in the main chain of glucan and additional beta (1-->>6) branching points that cause their antitumor effect. Most of the clinical evidence for antitumor activity comes from the polysaccharides like lentinan, PSK (krestin), and schizophyllan. It was found that the effectiveness of high-molecular-weight glucans overwhelms than that of low-molecular-weight glucans.

Satoru Arata, Jun Watanabe, and Masako Maeda (2016) studied the actual effect and underlying mechanisms of the effect of continuous intake of an aqueous extract from *I. obliquus* on the suppression of Lewis lung carcinoma growth and spontaneous metastasis in mouse models [2]. After 3 weeks of continuous intake of the extract at a dose of 6 mg/kg/day, the authors obtained significant suppressive effects: in models of tumor-bearing mice, a 60% reduction in the tumor was observed, while in metastatic mice, the number of maligning nodules decreased by 25% compared to the control group. In addition, mice treated with *I. obliquus* extract showed not only tumor reduction, but also inhibition of vascularization.

Eid J.I., Das B. (2020) consider *Inonotus obliquus* to be one of the most powerful antioxidants in the world [8]. The authors note that the therapeutic effects of chaga components are well characterized only in vitro; the effects of development in vivo are not described in detail. Using the example of Danio fish, the authors identified the influence of *I. obliquus* on the cell cycle and apoptosis. Polysaccharides of chaga mushrooms, in addition to phenolic compounds, included xylulose, rhamnose, mannose, glucose, inositol, and galactose. Staining with the DNA-binding dye acridine orange showed that polysaccharides of *Inonotus obliquus* alleviate oxidative stress [8]. Flow cytometric analysis using H2DCFDA, which specifically binds to fragmented DNA in cells, showed significantly reduced levels of intracellular reactive oxygen species (ROS) ($p < 0.05$), which in turn reduced apoptosis in developing embryos. Analysis of the cell cycle by measuring the DNA content using flow cytometry showed that chaga polysaccharides moderately delay cells at the G1 stage, thereby inhibiting cell proliferation, which can be further studied in cancer research. In general, temporary

exposure to chaga polysaccharide extract reduced the volume of intracellular reactive oxygen species (ROS) and contributed to the normal development of Danio fish.

Li Z., Mei J., Jiang L., Geng C., Li Q., Yao X., Cao J. (2019) found that tacrine, the first drug licensed for the treatment of Alzheimer's disease, induces apoptosis in HepG2 cells by generating reactive oxygen species (ROS) and mitochondrial dysfunction [18]. The authors studied the possible protective effect of polysaccharides from *I. obliquus*, obtaining evidence that *I. obliquus* polysaccharides reduce tacrine-induced apoptosis in HepG2 cells. The experiment also observed inhibition of tacrine-induced ROS generation, 8-OHdG formation in mitochondrial DNA, and loss of mitochondrial transmembrane potential when using *I. obliquus* polysaccharides. In addition, the intake of *I. obliquus* extract reduced the release of cytochrome C and activation of caspase-3 induced by tacrine. These data suggest that fungal polysaccharides may inhibit tacrine-induced apoptosis in HepG2 cells. This protection is mediated by an antioxidant cytoprotective mechanism [18]. Similar hepatoprotective properties of *I. obliquus* extract were proved by studies of Hong K.B., Noh D.O., Park Y., Suh H.J. (2015) [10].

Nguyen T.M.N., Le H.S., Le B.V. et al. (2020) note that the lanostane triterpenoid, inotodiol, is found exclusively in the chaga mushroom; the authors found evidence that purified inotodiol has activity to suppress mast cell function in vivo [23]. Inotodiol also relieved symptoms similar to those observed in the ovalbumin-induced (cOVA) mouse food allergy model. As with untreated 70% ethanol extract of chaga mushroom (320 mg/kg), oral administration of inotodiol (20 mg/kg), both for prevention and treatment, was accompanied by a significant reduction in the manifestations of allergic symptoms and inflammatory lesions in the small intestine that appear after repeated oral administration of cOVA. Despite the fact that inotodiol (20 mg/kg) and chaga mushroom extract (320 mg/kg) acted to the same extent, it was found that the immunological mechanisms underlying these effects differ from each other. The authors illustrated the evidence obtained from several in vivo analyses, including experiments with passive systemic anaphylaxis mediated by mast cells, activation and proliferation of adaptively transferred antigen-specific T cells, and production of IgG1, IgE, and IgA immunoglobulins by antigen-specific B cells. Inotodiol selectively inhibited mast cell function without significantly affecting other immune responses, while crude chaga mushroom extract randomly suppressed a variety of immune responses. This indicates the presence of several immunomodulating biologically active components in the chaga mushroom that act on various targets. The strong anti-allergic activity of inotodiol, as well as its remarkable selectivity against mast cells, makes it an excellent tool of choice for the treatment of food allergies with high efficiency and no side effects.

Many authors have described the positive antitumor effect of *I. Obliquus* and its extracts containing the trioterpenoid inotodiol. Nikitina S.A., Khabibrakhmanova V.R., Sysoeva M.A. (2016) summarized data on the chemical composition of triterpene and steroid compounds isolated from chaga mushroom grown in natural conditions or in synthetic culture [25]. Particular attention was paid to the biological activity of chaga mushroom extracts and these specific compounds against various cancer cell lines in vitro and in vivo. This analysis demonstrated some common features in the inhibition of growth of various cell lines by components of the chaga fungus. In this context, triterpene compounds containing an OH group at C22 and a side chain unsaturated bond are the most active [25]. Zhang P., Cao X., Li C., et al. (2016) studied the role of the squalene synthase gene isolated from *Inonotus obliquus* in triterpene synthesis by detecting squalene in vitro in a reaction mixture of particles using high-performance liquid chromatographic analysis [34]. The positive effect of treatment with the use of triterpenoid inotodiol have been demonstrated in hepatoma, leukemia, colon cancer and cervical cancer. Anti-carcinogenic effects of cell growth inhibition in lung carcinoma have also been shown. Due to the identified oncostatic activity of *I. obliquus*, Sun Y., Yin T., Chen X. H., Zhang G., et al. (2011) suggested using polysaccharide-triterpenoid complexes of *I. Obliquus* as components of heterogeneous inhibitors of cancer cell proliferation [28]. Zhang S.D., Yu L., Wang P. Kou, Li J. et al. (2019) studied the potential mechanisms of inotodiol for HeLa cell migration, invasion and apoptosis via the p53-dependent pathway, transwell invasion, flow cytometry, caspase-3 activity analysis and Western blot analysis, as well as the participation of the p53 signaling pathway in anti-metastatic and Pro-apoptosis [35]. In addition, the function of the p53 tumor suppressor was further verified by small interfering RNA. The dependence of the effect of inotodiol on its concentration was established. The cytotoxic effect on HeLa cells at concentrations above 25 microns significantly inhibits the growth of HeLa cells and even induces apoptosis. This result was further confirmed by analysis of cell proliferation and morphology. Analysis of in vitro wound healing and transwell invasion showed that treatment with inotodiol in low concentrations significantly inhibits cell migration and invasion in a dose-dependent manner.

Reduced levels of matrix metallopeptidase-2 (MMP2) and matrix metallopeptidase-9 (MMP9) also depend on the drug concentration. Inotodiol significantly induces apoptosis of tumor cells through its effect on annexin-V-FITC, which is associated with activation of pro-apoptotic PARP proteins, partition of caspase-3 and Bax expression, and inhibition of the anti-apoptotic Bcl-2 protein expression. The antitumor activity of inotodiol can be inhibited by switching off the tumor suppressor p53. Pretreatment of p53-specific small interfering RNA (si-p53) significantly inhibits inotodiol-induced apoptosis of HeLa cells and reduces the activity of caspase-3. Moreover, the inhibitory effect of inotodiol on tumor migration and invasion was blocked by p53 knockdown.

Zhang X., Bao C., Zhang J. (2018) investigated the effect of inotodiol (lanostane triterpenoid) on the cellular growth of breast tumors in diabetic conditions [36]. The research was conducted on a model of breast cancer against the background of diabetes in female Sprague-Dowley rats, caused by the administration of streptozotocin (STZ) at a dose of 35 mg/kg, followed by the induction of breast cancer 7,12-dimethylbenzanthracene (DMBA) at a dose of 10 mg/kg. Histological examination of the pancreas and of breast tumor proliferation was conducted by immunohistochemical methods with PCNA staining, Tunel method for apoptosis. The results of the study showed that inotodiol reduces blood glucose levels in SD rats, as well as reduces the level of cholesterol, triglycerides and high-density lipoproteins in blood plasma. Expression of the PCNA proliferation marker was reduced when taking inotodiol. It lowered the expression of β-catenin and its downstream targets (c-Myc and cyclin D1) with subsequent induction of apoptosis. Evidence-based results indicate that inotodiol regulates blood glucose levels in diabetic rats, participates in regulating of proliferation while inhibiting breast tumor progression, inducing apoptosis through reduced regulation of β-catenin signaling [36].

Anti-carcinogenic effect with targeted effects of *I. obliquus* extracts are shown against several cancer cell lines in the absence of cytotoxic effects to normal cells. The optimal concentration of these complexes was estimated at 150 mg/ml. Results of oral doses of *I. obliquus* polysaccharide complexes showed suppression of melanoma cell growth. Antihyperglycemic and antioxidant effects were demonstrated in experiments on mice with alloxan diabetes; data were obtained on lowering blood cholesterol levels and pancreatic regeneration. A number of studies have demonstrated the antimicrobial activity of unknown components of *I. obliquus*, which also have a clear bactericidal effect on a number of strains of Mycobacterium smegmatis and Francisella tularensis. Based on these properties, dental films based on nano-particles of chaga have been developed for the treatment of inflammatory bacterial diseases of the oral mucosa.

Chaga mushroom glycans are considered pathogenic associated molecular patterns (PAMP), similar to molecules that bind to specific pattern recognition receptors (PRRs) on the surface of immune cells. This interaction induces activation of a signaling cascade that directs the synthesis of a specific repertory of immune effector molecules [30]. Wasser S.P. (2011, 2014, 2017) noted the importance of studying these natural sources as a means of treating cancer [31]. Many fungal biopolymers have immunotherapeutic properties, contributing to the inhibition of growth and inducing the destruction of tumor cells. Although the mechanism of their antitumor action is still not fully understood, the development of methods for stimulating and modulating key immune responses with fungal polysaccharides is a central task in medicine in general and in oncology in particular [12]. Some of the fungal polysaccharides have passed through phase I, II, and III clinical trials and are widely and successfully used in Asia for the treatment of various types of cancer and other diseases [26]. Based on the chemical composition, it is assumed that the components of the chaga mushroom have 126 therapeutic functions, including antitumor, immunomodulatory, antioxidant, antiischemic, cardiovascular, antihypercholesterolemic, antiviral, antibacterial, antiparasitic, antifungal, detoxifying, hepatoprotective and antidiabetic effects [32]. Given that the mechanism of their antitumor action has not yet been fully studied, the issues of stimulation and modulation of immune responses by chemical components of chaga are of key relevance at the present stage [29].

The results of more than 600 clinical studies testing the effects of fungi on humans have been published in numerous papers [21]. Mushrooms are an extensive and at the same time almost inexhaustible source of powerful new pharmaceutical products [25]. In particular, and this is the most important thing for modern medicine, they are an unlimited source of polysaccharides [4]. These polysaccharides have different chemical compositions, and most of them belong to the group of beta-glucans; they have beta (1-->3) bonds in the main chain of glucan and additional beta (1-->>6) branching points that provide mechanisms for their antitumor action. It appears that high-molecular-weight glucans are more effective than low-molecular-weight glucans [6]. The same opinion is shared by Xin X., Qu J., Veeraraghavan V.P., (2019), who showed existing achievements in research on the mechanisms of isolated fungal polysaccharides,

in particular (1-->3) -β-D-glucans, which can cause various cellular responses, such as the expression of cytokines and nitric oxide, which carry out antitumor mechanisms indirectly through stimulating T cells or binding other molecules of cellular immunity, such as polypeptides and proteins, conjugation of which always has a strong antitumor activity [32].

Cytotoxic bioactivity of *I. obliquus* was established in four human lung adenocarcinoma cell lines with different p53 status (A549, H1264, H1299, and Calu-6) [22]. According to the authors, the analysis of apoptosis induction tests with MeOH *I. obliquus* extract showed a decrease in cell viability in all lung cancer cell lines, and the cytotoxicity of these compounds was mediated by apoptosis through activation of caspase-3. Directed increase in bioactivity through fractionation of MeOH extract and chemical study of its cytotoxic hexano-soluble and Ch2Cl2-soluble fractions led to the isolation of eight triterpenoids, including a new lanostane-type triterpenoid called chagabusone A. The structures of the isolates were elucidated by high-resolution spectroscopic analysis. Among the isolated compounds, some of them showed the most powerful cytotoxic activity in all studied human lung cancer cell lines, with IC50 values varying from 75.1 to 227.4 microns.

In the treatment of cancer, fungi can inhibit metastasis due to the presence of arctigenin, lignan, in its composition—an important component and a representative of the group of phenolic compounds of plant origin. As is known, the basis of tissue malignancy inhibitors, cytostatics, contains organic compounds of the aromatic series, in whose molecules the hydroxyl groups OH– are bound to the carbon atoms of the aromatic ring. Arctigenin refers to phytoestrogens that are metabolized in the same way as estrogen hormones. The use is accompanied by a reduction in the risk of breast cancer after menopause, which is due to the fact that lignans have a milder effect on cells than human estrogen, effectively blocking the action of powerful endogenous human estrogen [27]. At the same time, the probability of malignancy in the mammary gland, which depends on growth hormones, is reduced by inhibiting individual proteins (NPAT proteins) necessary for the formation of cancer cells, paralyzing the ability of maligning cells to reproduce. Studies have shown that arctigenin is effective in the treatment of lung, liver, and stomach cancer [19].

Kang J.H., Jang J.E., Mishra S.K., et al. (2015) studied the effect of various fractions and components of the chaga mushroom (*Inonotus Obliquus*) on viability and apoptosis in human colorectal cancer (CRC) cell lines, and revealed the most effective inhibition of tumor growth by a single component identified by pulsed nuclear magnetic resonance (NMR) as ergosterol peroxide with known antiproliferative and apoptotic activity [13]. Lee H.S., Kim E.J., Kim S.H. (2015) cultured human colon cancer HT-29 cells in the presence of 2.5–10 micrograms/ml of *I. obliquus* ethanol extract (IOEE) and analyzed cell cycle arrest by flow cytometry and Western blot protein expression control. It was found that the treatment of cells with 2.5–10 micrograms/ml of IOEE reduces the number of viable HT-29 cells and DNA synthesis, increases the percentage of cells in the G1 phase, reduces the expression of CDK2, CDK4 and cyclin D1 proteins, increases the expression of p21, p27 and p53, and inhibits phosphorylation of Rb and E2F1 expression. Among *I. obliquus* fractions, fraction 2 (fractionated with dichloromethane) showed the same effect as EEIO treatment on cell proliferation and the level of protein associated with the cell cycle [16].

Currently, *I. obliquus* is considered a non-specific medicine for gastritis, gastric ulcer, polyposis and is used for precancerous therapy in liquid or tablet form, as a complex drug "Befungin" represented by a concentrated extract of chaga mushrooms. To establish the pharmacological properties of *I. obliquus*, studies were conducted showing the anti-ulcer activity of ethanol extract of chaga. The anti-ulcer activity of *I. obliquus* was determined in rats with gastric ulcer (ethanol-induced ulcer). Ethanol extract of *I. obliquus* (200 mg/kg) did not cause any signs of toxicity or sensitivity in rats when administered orally. Oral administration of ethanol extract of *I. obliquus* showed anti-ulcer activity in all models used. The ethanol extract of *I. obliquus* showed effective anti-ulcer activity, which could be due to the presence of various biologically active compounds.

Na H.G, Park Y., Kim M.A., et al. (2019) noted that the use of chaga has numerous health benefits in alternative medicine in the treatment of obesity. Their proposed powder mixture of Chaga mushroom extracts was fermented using Lactobacillus acidophilus KCTC3925 (FCC), with the determination of an anti-cholesterol effect in a high-fat diet (HFD) in mice [20]. Analysis of the experimental results showed that the level of serum GT, GPT, and leptin, as well as the expression of hepatic COX-2 mRNA, as well as splenic COX-2 and IL-4 mRNA, were significantly higher in the HFD groups than in the control group ($P > 0.05$). With the exception of splenic IL-4 levels, these increases were significantly reduced by the addition of FCC. Expression of ICAM-1, a marker of aortic inflammation, was significantly increased in the HFD group. FCC suppressed weight gain and epididymal fat appendages, as well as inflammatory responses in the liver and spleen of HFD-fed mice.

Kazumi Sagayama, Naonobu Tanaka, Takatoshi Fukumoto, Yoshiki Kashiwada (2019) identified, after studying the effect of *Inonotus obliquus* on the proliferation of human papillary follicle cells (Hfdpc), five lanostane-type triterpenes (1-5) using spectroscopic data, of which lanosterol (1), inotodiol (3), lanost-8,24-diene-3β, 21-diol (4) and trametenolic acid (5) showed a more potent proproliferative effect on Hfpdc than minoxidil, an anti-alopecia agent used as a positive control. Lanostane triterpenes (1, 3, 4, and 5) were potential candidates for new products that can be used for hair care with a stimulating effect on hair growth [27].

Devi K.S. and Maiti T.K. (2016), in a review of patents for the extraction of biologically active substances (BAS) from chaga mushroom, showed that for decades the use of components from mushrooms was considered in the framework of ethnic medicine and was widely used in the treatment of various serious diseases [6]. Methods of extraction of pharmacologically significant fungal glucans affect their immunostimulating properties, structure, composition, solubility in water and conformation in solution. In addition, modifications of glucans depending on the particle size also contribute to a significant increase in their activity [15].

In order to increase the antitumor activity of polysaccharides and improve their clinical properties, which depend mainly on their solubility in water, the main procedures used to optimize the isolation of polysaccharides are Smith decomposition (redox hydrolysis), formolysis, and carboxymethylation [31].

Gil Y.G., Kang S., Chae A. et al (2018) developed and synthesized anisotropic porous palladium nanoparticles with full-wavelength absorption in the ultraviolet–visible–near-infrared range using concentration-dependent reduction synthesis to maximize the pharmacological activity of chaga mushroom (*Inonotus obliquus*) extract [9]. Porous Pd nanoparticles with chaga extract showed a surface antitumor effect, controlled delivery of doxorubicin via electrostatic interaction, and photothermal conversion effect under 808 Nm laser irradiation. The combined use of three approaches to cancer treatment has clearly demonstrated the feasibility of synergistic trimodal therapy. A modern platform using Pd, which is a key component of nanocatalysts, while not often used in biological applications, involves numerous applications using PD nanostructures, as well as the potential for development of new cancer treatments.

Kutaiba Ibrahin Alzand, Sabri Ünal, Mansor S. Mostafa Boufaris (2018) isolated three new lanostane-type triterpenes, a new abietane-type diterpenes, and 10 known compounds (5-14) from sclerotia of *Inonotus obliquus* [1]. Their structures were established using a combination of spectrometric methods, including infrared, 1-dimensional and 2-dimensional nuclear magnetic resonance and high-resolution electrospray mass spectrometry. During in vitro studies, the compounds showed hepatoprotective effects against D-galactosamine-induced damage in WB-F344 cells with inhibitory effects ranging from 35.4% to 83.8%. Some of the compounds showed selective cytotoxicity against Bel-7402, A549 or KB cell lines and inhibitory action against protein tyrosine kinases with half-maximum inhibitory concentrations of 23.8 and 7.4 mmol/l, respectively.

A simpler way to prepare the extract is to extract it from crushed fruit bodies of the fungus by boiling them in water (0.5 l) for 30 minutes. The yield of the extract from mushrooms will be 30% on average.

In the same volume of ethanol, an alcohol extract is prepared from the crushed fruit bodies of the fungus *I. obliquus* (0.5 l) for 5 days. The yield of the extract from mushrooms is 60% on average. The absence of side effects of the use of *I. obliquus* ethanol extract (200 mg/kg) and of signs of toxicity or sensitivity in rats after oral administration of *I. obliquus* ethanol extract with anti-ulcer activity in all models used is due to the presence of various biologically active compounds [14].

CONCLUSION

Water and alcohol extracts of *I. obliquus* that inhibit the proliferative activity of malignizing cells suggest that these fungi can potentially be used as an easily accessible source of natural antioxidants and inhibitors of the process of carcinogenesis and metastasis with promising potential in the prevention of metabolic disorders associated with reactive oxygen species (ROS). *I. obliquus* should be considered as a source for the manufacture of drugs with antioxidant, antimicrobial and antihyperglycemic activity. The most important component of chaga is betulinic acid, which ranks first in the ORAC for the level of antioxidants in food. The expressed activity of iron recovery dictates the study of the antiviral properties of the fungus *I. obliquus*, which is especially important at the present stage for the upcoming solution of issues with therapeutic and anti-epidemic measures against COVID-19. The results are encouraging, they explain and confirm the possibility of nutritional use of mushroom fruit bodies for the prevention and treatment of oxidative damage in neurodegenerative diseases, oncological pathology of tumors of various genesis and localization, as well as diseases of the pancreas and liver as hepatoprotective agents, and also as anti-allergic agents with a mild sedative effect. Chaga mushroom extracts can prevent

the development of breast, ovarian, cervical, prostate, lung, stomach, spleen, brain, and thymus cancer in the initial stages, as well as leukemia, lymphoma, and melanoma.

The effectiveness of the drugs made from extracts of the birch chaga mushroom (*Inonotus obliquus*) on tumors of various localization is high and allows us to conceptually justify the clinical application for patients with cancer. Glucan and triterpenoid components of the fungus allow for the use of *I. Obliquus* in some cases as a direct antitumor agent and a regulator of apoptosis. The use of polysaccharides from *I. obliquus* can actually prevent tacrine-induced hepatotoxicity. Analysis of the results of numerous studies provides experimental evidence confirming the prospects for the use of *I. obliquus* in the treatment of lung cancer, and reveals the molecular basis underlying its cytotoxic activity against human cancer cells. Inotodiol obtained from *I. obliquus* can be used for the prevention and treatment of not only chronic conditions such as diabetes but also breast cancer.

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