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# *Inonotus obliquus* – from folk medicine to clinical use

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ABSTRACT

The *Inonotus obliquus* (*I. obliquus*) mushroom was traditionally used to treat various gastrointestinal diseases. For many years, mounting evidence has indicated the potential of *I. obliquus* extracts for treatment of viral and parasitic infections. Furthermore, substances from *I. obiquus* have been shown to stimulate the immune system. The most promising finding was the demonstration that *I. obliquus* has hypoglycemic and insulin sensitivity potential. This review summarizes the therapeutic potential of *I. obliquus* extracts in counteracting the progression of cancers and diabetes mellitus as well as their antiviral and antiparasitic activities and antioxidant role. As shown by literature data, various authors have tried to determine the molecular mechanism of action of *I. obliquus* extracts. Two mechanisms of action of *I. obliquus* extracts are currently emerging. The first is associated with the broad-sense impact on antioxidant enzymes and the level of reactive oxygen species (ROS). The other is related to peroxisome proliferator-activated receptor gamma (PPARγ) effects. This receptor may be a key factor in the anti-inflammatory, antioxidant, and anti-cancer activity of *I. obliquus* extracts. It can be concluded that *I. obliquus* fits the definition of functional food and has a potentially positive effect on health beyond basic nutrition; however, studies that meet the evidence-based medicine (EBM) criteria are needed.


## 1. Introduction

*Inonotus obliquus* (*I. obliquus*) (Ach. ex Pers.) Pilát, belonging to the family *Hymenochaetaceae*, is a black-brown plant parasitic fungus.[1,2] This mushroom has also at least a few common and regional names such as [*Pol.*] czerniak brzozy, czyreń, czernidło, czarcie oko, or czanga, which most likely has been coined from the Russian word "chaga".[3] The genus *Inonotus* is widespread in North America, Asia, and Europe and includes approximately 100 species.[3,4] In Europe, this genus is represented only by 4 species, with *I. obliquus* as one of them.[4] Although it is widely distributed in North America, Asia, and Europe, *I. obliquus* is on the list of partially protected species of mushrooms in Poland.[5]

*I. obliquus* is a primary tree parasite causing decomposition of live trunks.[6] It has been seen on many trees species such as alder, beech, maple, rowan, hornbeam, poplar, oak, ash, willow, plane-tree, chestnut, and walnut, but the main hosts of *I. obliquus* are various species of birch.[3,7] It should be noted that the reports on the occurrence of *I. obliquus* on different species of deciduous trees are not reliable, because other fungi of the genus *Inonotus* are very often confused with the analyzed species.[3,7]

*I. obliquus* infects approximately 30–50-year-old trees through wounds in the bark and can grow on the trunk for another 30–80 years.[3,5] A few years after penetrating the trunk of live trees, it produces sclerotia (vegetative or asexual fruiting bodies) with a lumpy irregular shape, cracked surface, and black-brown color. The interior of the sclerotium itself is made of rust-brown, yellow-veined, very dense mycelium.[3] The sclerotia grow very slowly, reaching a diameter of more than 10 cm after 10–15 years.[5] On old trees, the growths can exceed 50 cm in diameter.[3] After many years, the host tree dies and annual fruiting bodies of the sexual stage appear. Fruiting bodies of this stage develop in the warm season of

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**Abbreviations**

| | |
|---|---|
| ABTS | 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) |
| ALT | alanine aminotransferase |
| AST | aspartate aminotransferase |
| Bax | Bcl-2-associated X protein B-cell |
| Bcl-2 | B- cell lymphoma 2 |
| CAT | catalase |
| COX-2 | cyclooxygenase-2 |
| DPPH• | 2,2-diphenyl-1-picrylhydrazyl |
| GPx | glutathione peroxidase |
| $H_2$DCFDA | 2′,7′-dichlorodihydrofluorescein diacetate |
| HCV | Hepatitis C Virus |
| HO-1 | heme oxygenase-1 |
| IFNγ | interferon gamma |
| IL-12 | interleukin 12 |
| IL-1β | interleukin 1 beta |
| IL-2 | interleukin 2 |
| IL-4 | interleukin 4 |
| IL-6 | interleukin 6 |
| IL-10 | interleukin 10 |
| iNOS | inducible nitric oxide synthase |
| IκBα | inhibitor kappa B alpha |
| LPS | lipopolysaccharides |
| MDA | malondialdehyde |
| MMPs | matrix metalloproteinases |
| NF-κB | nuclear factor kappa-light-chain-enhancer of activated B cells |
| NO | nitric oxide |
| Nrf2 | nuclear factor erythroid 2-related factor 2 |
| p-AKT | phospho-protein kinase B |
| $PGE_2$ | prostaglandin $E_2$ |
| PI3k | phosphatidylinositol 3-kinase |
| p-mTOR | phospho-mammalian target of rapamycin |
| PPARγ | peroxisome proliferator-activated receptor gamma |
| ROS | reactive oxygen species |
| SOD | superoxide dismutase |
| STZ | streptozotocin |
| T2DM | type 2 diabetes mellitus |
| TGF-β | transforming growth factor beta |
| TIMPs | tissue metallopeptidase inhibitors |
| TLR2 | toll-like receptor 2 |
| TLR4 | toll-like receptor 4 |
| TNFα | tumor necrosis factor alpha |
| XOD | xanthine oxidase |

the year in places with the most advanced rot. They very rarely grow on live trees.[3,8] This type of fruiting bodies may have considerable sizes (up to 3–4 m in length and up to 50 cm in width) and are eaten very quickly by insects, which are assumed to be the main (besides the wind) spore-spreading vector of *I. obliquus*.[3]

Numerous scientific studies have shown that the some assumptions of folk medicine beliefs are reasonable. Mexican and European folk medicine plants have been tested on *in vivo* or *in vitro* models, e.g. *Jasminum fruticans*, *Mentha longifolia,* and *Artemisia absinthium* extracts, which show anthelmintic or antibacterial effects.[9,10] Given these examples, the search for new potentially active, natural compounds in folk medicine extends the present knowledge and help to find a promising cure to such prevalent diseases as cancers, parasitic infestations, or bacterial infections. Therefore, the usefulness of *I. obliquus* extract in medicine, e.g. in the treatment of diabetes and parasitic and viral infections, was evaluated. However, there has been no work so far, presenting the current scientific findings on *I. obliquus* in a concise and substantive form. Therefore, the aim of this work is to present the current state of knowledge of the biological properties of the *I. obliquus* mushroom and the potential possibilities of its medical use.

## 2. Application of *I. obliquus* in folk medicine

In folk medicine, rational premises are intertwined with elements of magic. The rational premises are based on observation of the natural environment and finding substances that alleviate disease symptoms. In many countries, this is the only way in which people can improve their health due to poverty in society or difficult access to scientific medicine. *I. obliquus* has been used in folk medicine since ancient times. One of the oldest documents confirming the use of its conks for medicinal purposes is the work by Hippocrates "Corpus Hippocraticum".[3] The father of medicine used infusions of this mushroom externally to wash wounds.

In Eastern Europe, the Chaga mushroom has been used since the 12th century. Historical sources describe healing of a lip tumor in a Kiev Kniaz.[11] *I. obliquus* was used in traditional medicine for many indications by the people of Siberia.[12] The fungus was applied due to its antiparasitic, anti-tuberculosis, anti-inflammatory, and gastrointestinal properties.[13] It was also recommended for heart and liver diseases. Most often, it was used in the form of infusions, inhalations, or aqueous macerates.[13] Also antiseptic soaps containing *I. obliquus* were prepared for external use. In the middle of the 20th century, Chaga infusions were used as a substitute for tea in Siberia.[14] Relatively early attention was paid to the potential *I. obliquus* antitumor or supportive effect in cancer treatment, which was particularly important before the era of scientific oncology. Such descriptions can be found in popular literature, e.g. in Aleksander Solzhenitsyn's Cancer Ward.[14]

In Asia (China, Japan, Korea), Russia, and the Baltic countries, extracts of Chaga mushroom were used due to their beneficial effects on the plasma lipid system and heart function as well as antibacterial, anti-inflammatory, and anti-cancer activity.[15] Furthermore, the antioxidant activity of *I. obliquus* may be important for prevention of free radical-related civilization diseases (atherosclerosis, cancer, diabetes, accelerated aging, and degenerative diseases of the central nervous system).[16] Chaga extracts have been shown to inhibit the reproduction of hepatitis C virus (HCV) and human immunodeficiency viruses (HIV).[17]

The only objective method to assess the effectiveness of any therapy in scientific medicine is to perform research that meets the criteria of evidence-based medicine (EBM). Unfortunately, no observations from folk medicine or folk tales meet these criteria. Therefore, while drawing information from these sources, it is necessary to conduct scientific research that will either refute or confirm the folk premises. Importantly, the highest EBM standards should be maintained during the research, giving grounds for verifying folk knowledge.

## 3. Chemical composition and approaches to extraction of substances from *I. obliquus*

Only young and fresh sclerotia growing on birches, harvested throughout the year, are used in medicine.[3] Sclerotia should be harvested in uncontaminated areas distant from sources of pollution, which may accumulate in the fungus.[18] In the natural

**Table 1**
Approaches to isolation and administration of extracts or various substances isolated from *Inonotus obliquus*.

| TYPES OF EXTRACTS FROM *Inonotus obliquus* TESTED | REFERENCES |
|---|---|
| ENTIRE WATER EXTRACTS | 28,30,31,39,41–45 |
| ENTIRE ETHANOL EXTRACTS | 1,30,36,41,42,46–48 |
| ENTIRE METHANOL EXTRACTS | 30,32,41,49 |
| ENTIRE SODIUM HYDROXIDE EXTRACTS | 41 |
| ENTIRE CYCLOHEXANE EXTRACTS | 31 |
| ENTIRE ETHYL ACETATE EXTRACTS | 31 |
| MELANIN FRACTION | 24,29,50 |
| INODOTIOL FRACTION | 33,51 |
| FLAVAN FRACTION | 34 |
| TRITERPENOIDS AND STEROIDS FRACTIONS | 1,35,52–54 |
| POLYSACCHARIDE FRACTIONS | 1,37,62–69,38,55–61 |
| POLYPHENOLIC FRACTION | 1,32,36,70 |

environment, *I. obliquus* grows in a cool climate with high seasonal temperature fluctuations, freezing, UV radiation, as well as occurrence of bacterial and viral infections.[19,20] In response to numerous stressors, *I. obliquus* has developed complex defense mechanisms. These mechanisms include the production of various bioactive substances: antioxidants, triterpenoids, ergosterol and its peroxide, sesquiterpenes, benzoic acid derivatives, hispidin analogues, and melanins. In addition, high expression of antioxidant enzymes has been detected in *I. obliquus*.[21–26] Moreover, many reports have highlighted that the polysaccharide fraction present in *I. obliquus* extracts is the largest group of active compounds, besides phenols. Therefore, many investigations have been performed to evaluate the polysaccharide content in this mushroom extracts, also in various conditions of extraction such as freeze, hot air, or vacuum drying methods.[27] As reported by Ma et al. *I. obliquus* extracts contain many low molecular polysaccharides, which are considered antioxidant agents responsible for this activity of these mentioned extracts.[27] This antiradical property has been repeatedly proved in the literature *inter alia* by Ma et al. or Cui et al. who tested the antioxidant properties of *I. obliquus* saccharides *in vitro* or *in vivo* on rat livers, respectively.[1,27] The water extract contained 19.76–26.51% of neutral sugars, in which the sugar composition varied depending on the drying method, but rhamnose, galactose, and glucose were the dominant sugars, whereas arabinose or mannose were the least abundant components.[27] The chemical structure of *I. obliquus* polysaccharides was also strictly related to the method applied, indicating that the saccharides were characterized by a dense spherical, branching, and elongated rod structure.[27]

Currently, many approaches for isolation of substances from *I. obliquus* have been described; they are summarized in Table 1. They can be divided into techniques yielding the whole of isolated substances, individual fractions, or even single active substances from *I. obliquus*.[1,28,29] The use of total aqueous or ethanol extracts is the most similar approach to the methods used in folk medicine.[28,30] These types of extracts are suitable for direct consumption. Unfortunately, the application of other methods of total extraction using organic solvents such as methanol, ethyl acetate, or cyclohexane prevents the use of such a product.[30–32] From a practical point of view, the use of a whole extract in scientific research is very important for practical natural or herbal medicine. In addition, many substances present in the whole extract can abolish or enhance each other's activity. On the other hand, it is not possible to investigate the exact mechanism of specific substances in a non-fractionated extract. Another approach is to use individual fractions obtained from *I. obliquus*. In the literature, there are descriptions of research on various substances isolated from *I. obliquus*, including melanin, inodothiol, flavans, triterpenoids, steroids, polyphenols, or polysaccharides.[1,24,33–36] This type of approach makes it possible to determine precisely which substance has therapeutic effects. Currently, the most frequently performed research is focused on the polysaccharide fraction (summarized in Table 1). As shown in the literature, this is a very promising group of substances associated with anti-cancer activity and insulin-improving sensitivity.[37,38] It should be noted that proteins contained in extracts are not studied. The extraction methods denature the protein at an early stage of isolation. In the case of aqueous extracts, the isolation of the substance is carried out at a temperature between 80 and 100 °C.[37,39,40] Similarly, various organic solvents (ethanol, methanol, cyclohexane, etc.) as well as multi-stage extraction methods can destroy the bioactive active ingredients contained in *I. obliquus*.

## 4. Biological properties of *Inonotus obliquus* extracts

Many different properties of extracts or substances derived from *I. obliquus* have been described to date. These include antiviral, antidiabetic, antioxidant, antiparasitic, immunomodulatory, anti-inflammatory, neuroprotective, anticancer properties *in vitro*, and recently antifatigue effects.[1,30,36,37,39,65,71] Relevant literature data are summarized in Table 2.

### 4.1. Antioxidant properties

The antioxidant properties of extracts and individual fractions of substances obtained from *I. obliquus* are the most frequently studied parameter in scientific publications. To determine the antioxidant potential, methods based on the stable 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•) are used.[1,57,61,77,85,96–98] Less often, another stable 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical is used.[70,79] The DPPH• and ABTS methods are based only on the antioxidant properties of chemical substances and do not fully reflect the antioxidative potential of a given extract or substance.[99,100] Methods based on the impact on the activity or expression of antioxidant enzymes as well as the formation of reactive oxygen species (or scavenging) in living cells reflect better the actual state in the organism. To date, the effects of *I. obliquus* extracts on the activity and/or expression of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), xanthine oxidase (XOD) in cell cultures and mouse tissues have been described.[30,37,55,57,59,66,78] In addition, extracts from *I. obliquus* affected reactive oxygen species (ROS) production measured with the malonic dialdehyde (MDA) and 2′,7′-dichlorodihydrofluorescein diacetate ($H_2$DCFDA) methods.[58,63,92] Interestingly, the effects were different depending on the type of cell. Szychowski et al. showed that extracts from *I. obliquus* reduced the amount of ROS in normal cells[30,58]; in turn, numerous research teams described an increase in ROS in cancer cell lines.[30,67]

Flavan derivatives, polysaccharides, and 3,4-dihydroxybenzalacetone isolated from *I. obliquus* have also been shown to have neuroprotective effects in neurodegenerative disease models such as Alzheimer and Parkinson diseases and the SH-SY5Y cell line.[34,66,92] These substances were found to protect against oxidative stress or increase SOD expression. Similarly, in other cells considered normal, such as pancreatic (RINm5F cell line) and hepatic (L02 cell line) cells, *I. obliquus* polysaccharides protected against $H_2O_2$-induced damage.[42,58,77]

### 4.2. Anti-inflammatory and immunomodulatory effects

So far, few research teams have tried to determine the impact of extracts obtained from *I. obliquus* on the immune system.[33,88,89] Fan et al. have shown that water-soluble polysaccharides obtained from

**Table 2**
Properties of different extracts from *Inonotus obliquus*. *In vitro* means a cell culture model or chemical analysis (cell-free model) *in vivo* means experiments performed on animals. To date, no human experiments have been performed.

| PROPERTIES OF DIFFERENT EXTRACTS FROM *Inonotus obliquus* | TYPE OF BIOLOGICAL MODEL USED | REFERENCES |
|---|---|---|
| ANTIVIRAL | *in vivo* | 17,39,72–74 |
| ANTIDIABETIC | *in vivo/in vitro* | 29,37,55,56,75,76 |
| ANTIOXIDANT/ROS SCAVENGER | *in vivo/in vitro* | 1,30,59,60,70,75,77–82,36,83–87,37,42,50,55–58 |
| ANTIFATIGUE | *in vivo* | 71 |
| ANTIPARASITIC | *in vivo* | 63–65 |
| IMMUNOMODULATORY | *in vivo/in vitro* | 33,48,61,62,64,88–90 |
| ANTI-INFLAMMATORY | *in vivo/in vitro* | 33,36,41,57,86,91 |
| NEUROPROTECTIVE | *in vivo/in vitro* | 34,66,82,92 |
| ANTI-CANCER POTENTIAL | *in vitro* | 28,30,49,51,63,67,75,93–95,31,32,38,43–47 |

*I. obliquus* increased the proliferation of murine peritoneal lymphocytes.[38] Polysaccharides from *I. obliquus* also stimulated the production of tumor necrosis factor-alpha (TNFα) by mouse macrophages. TNFα production increased in direct proportion to the increasing concentration of the tested polysaccharides.[38] Similarly, Chen et al.[101] observed that polysaccharides from *I. obliquus* increased the proliferation of splenocytes and lymphocytes. In addition, *I. obliquus* increased the secretion of cytokines such as interleukin-2 (IL-2), interleukin-6 (IL-6), interleukin-12 (IL-12), and TNFα and intensified the phagocytic processes. *I. obliquus* also significantly increased Bax expression and inhibition of Bcl-2 [101]. In turn, the latest research has shown that *I. obliquus* extracts very strongly reduced TNFα secretion in the RAW 264.7 cell line.[41] Similarly, Chen et al. showed that polysaccharides from *I. obliquus* affected the Th1/Th2 lymphocyte ratio and Th17/Treg in the colon, mesenteric lymph nodes, and spleen of male BALB/c mice.[89] Using a mouse model, the authors showed that the studied polysaccharides from *I. obliquus* could be used to treat inflammatory bowel disease. Other studies performed on BALB/c mice showed that extracts from *I. obliquus* suppressed Th2 and Th17 immune response.[33] It is currently thought that *I. obliquus* extracts or their polysaccharide fraction inhibit inflammatory reactions.[41,57] Most studies show a reduction in the production and/or secretion of proinflammatory cytokines such as interleukin-1 beta (IL-1β), interferon gamma (IFNγ), and TNFα.[41,57,89] *In vivo* studies showed that an *I. obliquus* extract administered to Sprague-Dawley rats for 7 days protected the animals against the development of induced inflammation. The same studies showed that the *I. obliquus* extract exhibited analgesic properties as well.[16] Park et al. tried to elucidate the mechanism of the anti-inflammatory and analgesic effects of *I. obliquus* extracts on the RAW 264.7 cell line.[16] It was demonstrated that the extract caused a decrease in the production of nitric oxide (NO) and prostaglandin $E_2$ ($PGE_2$).[16] In addition, LPS-stimulated macrophages produced nitric oxide synthase (iNOS), which was inhibited by the *I. obliquus* extract. Furthermore, the tested extract inhibited lipopolysaccharide (LPS)-induced cyclooxygenase-2 (COX-2) production.[16] The *I. obliquus* extract also reduced mRNA production and expression of TNFα and the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) induced by LPS.[16] Recent studies have shown that individual fractions from *I. obliquus* can act antagonistically. Wold et al. proved that some polysaccharide fractions increased NO production by murine macrophage and dendritic cell lines J774. A1 and D2SC/1 [62]. It cannot be excluded that, depending on the type of cells tested, the extracts or individual substances from *I. obliquus* may increase or inhibit NO production.

### 4.3. Antiviral, antibacterial, and antiparasitic properties of *I. obliquus*

Data on the antiviral activity of *I. obliquus* extracts are very limited. To date, it has been shown that polysaccharides derived from the aqueous fraction of *I. obliquus* inhibit the protease from HIV type 1 (HIV-1), and thus impede the entry of virions into cells.[39] In other studies, Shibnev et al. showed that the *I. obliquus* water extract was active against the HCV.[17] At 48 h after addition of the *I. obliquus* aqueous extract to the HCV-infected embryonic porcine kidney epithelial inoculated line (SPEV), the amount of the virus in the cells was inversely proportional to the concentration of the added extract. At the highest concentrations, the extract completely stopped or significantly inhibited the reproduction of HCV. Moreover, it was shown that the addition of the extract to the cells 24 h before their exposure to the virus protected the SPEV cells from infection.[17] In another experiment, an aqueous extract of *I. obliquus* showed an effect against the herpes virus - Herpes simplex type 1 (HSV-1) in normal kidney cells (Vero) of infected *Cercopithecus aethiops*.[102] The presented studies confirm the antiviral effect of *I. obliquus* and indicate its potential use in the treatment of diseases associated with viral infections. In addition, the polysaccharide fraction of *I. obliquus* exhibited a broad-spectrum antiviral activity against feline herpesvirus 1, feline influenza virus H3N2 and H5N6, feline panleukopenia virus, and feline infectious peritonitis virus, which cause respiratory and gastrointestinal diseases in cats.[73] Similarly, Seo and Choi showed that ethanolic extracts from *I. obliquus* inhibited the entry of murine norovirus (MNV) and feline calicivirus (FCV) into RAW264.7 cells [72].

In the available literature, there are only two reports suggesting the antibacterial or probiotic effect of *I. obliquus* extracts. Niu et al. showed that various fractions derived from *I. obliquus* stimulated NO production and additionally increased phagocytosis in RAW 264.7 cells.[61] As demonstrated by Hu et al. *I. obliquus* polysaccharide was found to regulate the gut microbiota in chronic pancreatitis in mice.[103] Moreover, the authors suggest that administration of *I. obliquus* polysaccharides could regulate the gut microbiota composition and diversity to a healthy profile in mice with chronic pancreatitis.

To date, only one team has studied the antiparasitic aspects of *I. obliquus* extracts and polysaccharides [63–65]. *Toxoplasma gondii* (*T. gondii*) is an obligate intracellular protozoan that causes toxoplasmosis in humans and many warm-blooded animals. Approximately 30%–50% of the population is infected with *T. gondii* worldwide.[104] Xu et al. described that *I. obliquus* polysaccharides significantly reduced the abortion rate, inhibited the decreases in progesterone (P) and estriol (E3) levels and the increase in the MDA level, and increased the activities of SOD and GSH.[64] Furthermore, *I. obliquus* polysaccharides inhibited the production of inflammatory cytokines, such as TNFα, IL-6, IFNγ, IL-1β, and IL-17A, and promoted the production of the anti-inflammatory cytokine interleukin-10 (IL-10) and transforming growth factor (TGF)-β in *T. gondii*-infected pregnant mice.[64] Similarly, Xu et al. described that *I. obliquus* polysaccharides significantly decreased the liver coefficient and the levels of alanine aminotransferase (ALT),

**Table 3**
Anti-cancer potential of extracts or substances isolated from *Inonotus obliquus* tested on different tumor cell lines.

| CANCER CELL LINE | ASSAYS | REFERENCES |
|---|---|---|
| **A549** human lung adenocarcinoma | LDH release | 28,95 |
| | Caspase-3 activity | 67,95 |
| | DNA fragmentation | |
| | Cell viability | 28,32,49,67,68,95 |
| | Cell migration | 28 |
| | Cell proliferation | |
| | ROS production | 67 |
| | Cytotoxicity | 31 |
| **HeLa** human cervical cancer | Cell viability | 49,51 |
| | Caspase-3 activity | 51 |
| | Cell migration | |
| **4T1** mouse mammary carcinoma | Cell viability | 47 |
| **MCF**-7 human breast cancer | Cell viability | 47,49,68 |
| **AGS** human gastric cancer | Cell viability | 49 |
| **HEK-293** human embryonic kidney | Cell viability | 49 |
| **PA-1** human ovarian teratocarcinoma | Cell viability | 32 |
| **U937** human myeloid leukemia cell | Cell viability | 32 |
| **HL-60** human myeloid leukemia | Cell viability | 32 |
| **CACO-2** human colon carcinoma | Cell viability | 30 |
| | LDH release | |
| | ROS production | |
| | Cell proliferation | |
| **HT-29** human colorectal adenocarcinoma | Cell viability | 28,46 |
| | DNA synthesis | |
| | Cell proliferation | |
| | LDH release | 28 |
| | Cell migration | |
| **H1264** lung adenocarcinoma | LDH release | 95 |
| | Caspase-3 activity | |
| | DNA fragmentation | |
| | Cell viability | |
| **HCT-116** human colon carcinoma | Cell viability | 45 |
| | LDH release | |
| | Caspase-3 activity | |
| **H1299** human non-small cell lung cancer | LDH release | 95 |
| | Caspase-3 activity | |
| | DNA fragmentation | |
| | Cell viability | |
| **B16–F10** murine skin melanoma | Cell viability | 44,68,93 |
| | Caspase-3 activity | 44 |
| | Cell cycle inhibition | |
| | Cell migration | 93 |
| **Calu-6** human pulmonary adenocarcinoma | LDH release | 95 |
| | Caspase-3 activity | |
| | DNA fragmentation | |
| | Cell viability | |
| **HEC-1B** human endometrial adenocarcinoma | Cell viability | 68 |
| **P388** mouse leukemia | Caspase-3 activity | 94 |
| | Cell viability | |
| | DNA fragmentation | |
| **Fao** rat hepatoma | Cell viability | 28 |
| | Cell proliferation | |
| | LDH release | |
| | Cell migration | |
| **P19** mouse embryo teratocarcinoma | Cell viability | 28 |
| | Cell proliferation | |
| | LDH release | |
| | Cell migration | |
| **C6** rat glioma | Cell viability | 28 |
| | Cell proliferation | |
| | LDH release | |
| | Cell migration | |
| **Hep3B** human hepatoma | Cell viability | 43,107 |
| | Cell cycle inhibition | 43 |
| **HepG2** human hepatoma | Cell viability | 43,107 |
| | Cell cycle inhibition | 43 |
| | ROS production | 108 |
| **Hur7** human hepatoma | Cell viability | 68 |
| **KATO-III** human stomach carcinoma | Cell viability | 68 |
| **DLD-1** human colon carcinoma | DNA fragmentation | 40 |
| **SGC-7901** human gastric carcinoma | Cell viability | 38 |
| **SK-OV3** human ovary adenocarcinoma | Cell viability | 68 |
| **SW156** kidney adenocarcinoma | Cell viability | 68 |
| **SW620** human colorectal adenocarcinoma | Cell proliferation | 105 |

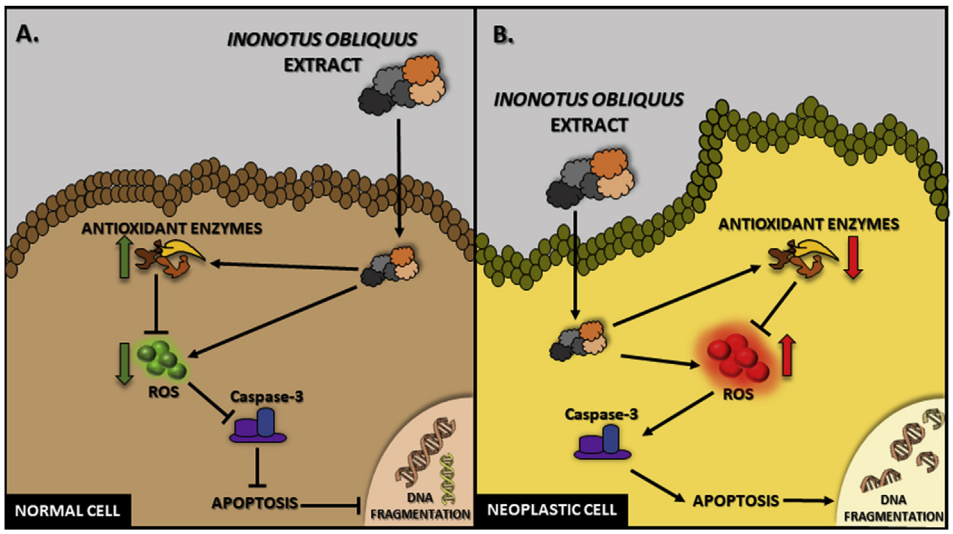


**Fig. 1.** Proapoptotic and antioxidant action of *Inonotus obliquus* extracts in normal and neoplastic cells described in literature.

aspartate aminotransferase (AST), MDA, and nitric oxide NO. In turn, the polysaccharides increased the levels of antioxidant enzymes SOD and GSH.[63] As mentioned previously, *I. obliquus* polysaccharides also effectively decreased the expression of serum TNFα, IL-6, IL-1β, IFNγ, and interluekin-4 (IL-4) in *T. gondii*-infected mice. The authors found that *I. obliquus* polysaccharides down-regulated the levels of toll-like receptor 2 (TLR2) and toll-like receptor 4 (TLR4) and phosphorylation of NF-κB p65 and inhibitor kappaBα (IκBα), but up-regulated the expression of nuclear factor erythroid 2-related factor 2 (Nrf2) and heme oxygenase-1 (HO-1).[63] Lastly, Ding et al. showed that *I. obliquus* polysaccharides significantly improved the spermatogenic capacity, ameliorated pathological damage to testis, and increased the levels of serum testosterone, luteinizing hormone, and follicular-stimulating hormone in *T. gondii*-infected male mice. *I. obliquus* polysaccharides effectively up-regulated the expression of testicular steroidogenic acute regulatory protein (StAR), P450scc, and 17β-HSD. These compounds further enhanced testicular phosphatidylinositol 3-kinase (PI3K), phospho-protein kinase B (*p*-AKT), and phospho-mammalian target of rapamycin (*p*-mTOR) expression levels.[65]

### 4.4. Anti-cancer potential

In medical databases, there are many publications on the anti-cancer activity of *I. obliquus* extracts *in vitro*. The anti-cancer potential of the extracts or substances isolated from *I. obliquus* and tested on different tumor cell lines are described in Table 3. Unfortunately, studies on animals are very limited and there are no reliable publications on humans.[57,105] Most studies have been conducted on cell lines originating from the digestive system, such as AGS, HCT-116, HT-29, SW620, SGC-7901, DLD-1, and CACO-2 [30,38,46,105]. Extracts from *I. obliquus* have been shown to inhibit proliferation and/or are cytotoxic to these human gastrointestinal tumor cell lines. Similarly, their cytotoxic, anti-proliferative, or proapoptotic effects have been demonstrated in cell lines from many other tissues and systems, e.g. lung, cervix, mammary gland, ovary, liver, lymphoid cancers (A549, MCF-7, HepG2, HepG3B, HeLa, MCF-7, HL-60, PA-1, H1264, H1299) (described in detail in Table 3).[28,32,43,49,94,95,106]

Unfortunately, many of these studies lack in-depth experiments. In some publications, the authors have limited themselves to reporting whether a given extract is toxic to cells without elucidation of the molecular mechanism of action, using only one parameter. These include investigations of 4T1, MCF-7, AGS, HEK-293, PA-1, U937, HL-60, P388, Hur7, HEC-1B, KATO-III, SG-7901, SK-OV3, and SW156 cell lines.[32,38,47,49,68] Interestingly, the majority of studies were conducted on the A549 line, which is a human lung adenocarcinoma line with the best documented anti-cancer potential of *I. obliquus* extracts or fractions of substances derived from the extract.[28,31,67,68,95] In publications elucidating the molecular mechanism of extracts, the authors consistently emphasize the role of ROS in the mechanism of action of substances derived from *I. obliquus*.[28,30] The authors also agree that extracts from *I. obliquus* intensify the process of apoptosis measured by capase-3 activity or the level of DNA fragmentation and stop the cell cycle in the G0 phase.[44,95,109] Very important in migration/metastasis of tumors are matrix metalloproteinases (MMPs).[110] Interestingly, it has been shown that, although the *I. obliquus* extract was not toxic to the A549 cell line at low concentrations, it statistically significantly reduced cell migration.[111] The authors attributed this effect to the declining MMP-2 and MMP-9 expression and the increasing expression of tissue metallopeptidase inhibitor-2 (TIMP-2) as well as the reduced expression of NF-κB.[111] Similarly, a decrease in NF-κB expression in A549 induced by *I. obliquus* extracts has been described.[109] Moreover, it has been reported that *I. obliquus* polysaccharides inhibit the expression of MMP-2, MMP-7, and MMP-9 in B16–F10 mouse cells.[93] Recently, Zhang et al. described that inotodiol from *I. obliquus* decreases the expression of MMP-2 and MMP-9 in HeLa human cells and reduces cell invasiveness.[51] Unfortunately, the tests performed *in vivo* are very limited. To date, it has been shown that tumors of the 3LL mouse lung cancer line implanted in C57BL/6 mice developed significantly more slowly in animals treated with an *I. obliquus* aqueous extract. In the experiment, it was shown that the tumor in mice receiving the extract was by 60% smaller and the number of metastases decreased by 25%. Interestingly, ingestion of *I. obliquus* reduced the body weight of the mice and increased their body temperature, which may have contributed to the protection against cancer, as suggested by the researchers.[112] Furthermore, *I. obliquus* extracts have been shown to inhibit the development of two tumor lines: melanoma B16–F10 and sarcoma-180 after implantation into Balbc/c strain mice.[44,49] It has also been shown that ergosterol isolated from *I. obliquus* inhibited the development of human colorectal cancer in the C57BL/6 mouse strain.[105] It is currently believed that the high

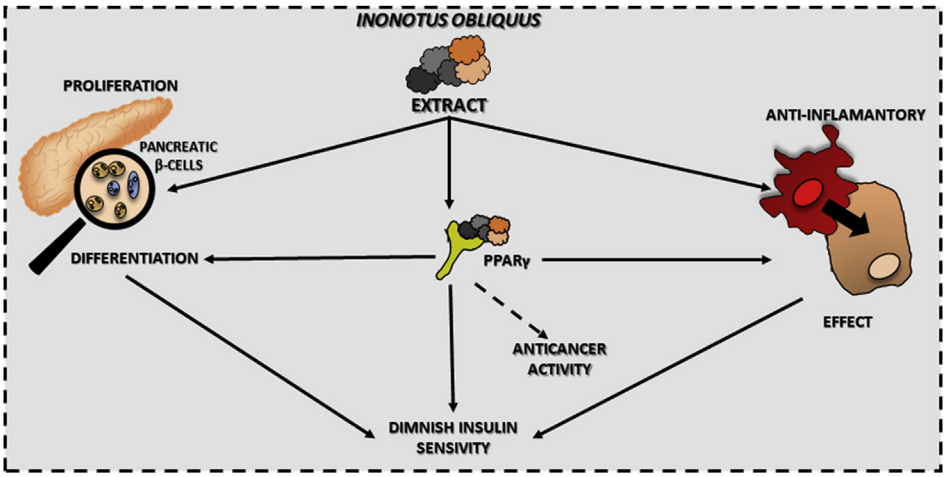


**Fig. 2.** Antidiabetic and anti-inflammatory action of *Inonotus obliquus* extracts with key involvement of the PPARγ receptor described in literature.

content of polysaccharides is responsible for the anti-cancer potential of *I. obliquus*.[67,111] However, the anti-cancer potential have been found in all types of extracts used.[113]

### 4.5. Hypoglycemic and insulin sensitivity action

ROS play a key role in many signaling pathways. Moreover, the relationship between oxidative stress and metabolic disorders such as type 2 diabetes (T2DM), cancer, obesity, and cardiovascular disease is well described.[114] To date, it has been shown that *I. obliquus* polysaccharides have hypoglycemic activity.[115,116] Unfortunately, the exact mechanism of action has not been elucidated. It has been described that *I. obliquus* polysaccharides in streptozotocin (STZ)-induced diabetic Wistar rats reduced blood glucose levels and restored the structure of β-cells after diabetes-induced cellular damage.[117] *I. obliquus* polysaccharides lowered the level of lipid peroxidation products (such as low-density lipoprotein), whereas the high-density lipoprotein cholesterol level was enhanced.[117] Moreover, Wang et al. reported that *I. obliquus* polysaccharides enhanced the serum levels of insulin and alleviated the metabolic derangement of glucose enzymes in STZ-induced diabetic mice.[56] In the same experimental model, *I. obliquus* polysaccharides with the chromium (III) complex significantly decreased fasting blood glucose levels, plasma insulin levels, and body weight in mice.[55] This is consistent with the previously described studies of total extracts. Cha et al. demonstrated that *I. obliquus* dietary treatment lowered serum glucose and leptin levels and alleviated obesity-related complications in T2DM OLETF rats.[118] Similarly, Sun et al. described that feeding the experimental mice with *I. obliquus* improved serum insulin levels, moderately expanded the pancreatic islets, and reduced pancreatic injuries in alloxan-induced diabetic mice.[119] In turn, *I. obliquus* polysaccharides elevated insulin levels in C57BL/6 mice with diabetic nephropathy; however, the cholesterol and triglyceride levels remained unaffected.[120] Recently, *I. obliquus* polysaccharide has been reported to help to alleviate pancreatic acinar atrophy and weight loss in chronic pancreatitis mice induced by diethyldithiocarbamate. Hu et al. postulated that *I. obliquus* polysaccharides possessed strong antioxidant activity for scavenging free radicals *in vitro* and *in vivo*, which could be beneficial for chronic pancreatitis therapy in mice.[57]

Peroxisome proliferator-activated receptor gamma (PPARγ) is a key receptor involved in insulin resistance and cell sensitivity to this hormone.[121] Moreover, PPARγ is also involved in anti-inflammatory action, by controlling activation and expression of NF-κB and pro-inflammatory cytokines.[122] However, only one paper showed that *I. obliquus* extract activated adipogenesis of 3T3-L1 preadipocytes, enhanced the expression PPARγ target genes, and increased triacylglycerol accumulation.[123]

## 5. Conclusions and perspectives

For decades, extracts of *I. obliquus* have been used as a remedy for numerous diseases in folk medicine. Recent discoveries have provided evidence of the appropriateness of using these drugs *in vitro*. Extracts from *I. obliquus* prepared in various solvents as well as individual fractions of substances derived from the fungus show antiviral, antibacterial, immunostimulating, and anti-tumor activity *in vitro*. To date, various studies have suggested significant therapeutic potential of substances derived from *I. obliquus*. Based on the literature data, it can be concluded that the effectiveness of extracts and/or substances derived from *I. obliquus* is based on two main mechanisms. The first one is the effect of *I. obliquus* on the production of ROS and/or on mechanisms of ROS scavenging, which varies depending on the cell type. The proposed scheme of the effect is described in Fig. 1.

The other mechanism is based on the action *via* the PPARγ receptor. Experimental evidence suggests that extracts and/or substances obtained from *I. obliquus* may affect the level of expression or activity of the PPARγ receptor.[123] In this way, extracts from *I. obliquus* can reduce insulin resistance. However, it cannot be excluded that the anti-cancer mechanism of action of *I. obliquus* extracts also involves the PPARγ receptor. The proposed scheme of the effects is described in Fig. 2. Unfortunately, due to the lack of data on the involvement of the PPARγ receptor in the anti-tumor activity of *I. obliquus* extracts, further research is required. It can be concluded that *I. obliquus* fits the definition of functional food and has a potentially positive effect on health beyond basic nutrition; however, studies that meet the EBM criteria are needed. Taking the above into account, we suppose that the *I. obliquus* extracts can be included in cancer therapies in the future; however, more specialistic analysis should be performed, especially on *in vivo* models.

## CRediT authorship contribution statement

**Konrad A. Szychowski:** Data curation, Formal analysis, Writing - original draft. **Bartosz Skóra:** Writing - original draft. **Tadeusz**

**Pomianek:** Writing - original draft. **Jan Gmiński:** Writing - original draft.

## Declaration of competing interest

The authors declare that there is no conflict of interest.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtcme.2020.08.003.

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