PRODUCT REFERENCE LIST

FENO-CHAGA™

Scientific publications relevant for Feno-Chaga™ line of products

APPLIES TO PRODUCTS	PRODUCT #
FENO-CHAGA™ M	20 097
FENO-CHAGA™	20 730
FENO-CHAGA™ L	20 728
FENO-CHAGA™ M ORGANIC	20 738
FENO-CHAGA™ L ORGANIC	20 731
FENO-CHAGA™ ORGANIC	20 729

NUTRACEUTICALS REFERENCES

103 references

COSMETICS REFERENCES

7 references

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ANTI-CANCER EFFECTS

Bioactivity-based analysis and chemical characterization of cytotoxic constituents from Chaga mushroom (Inonotus obliquus) that induce apoptosis in human lung adenocarcinoma cells.

J Ethnopharmacol. 2018 Oct 5; 224:63-75. doi: 10.1016/j.jep.2018.05.025. Epub 2018 May 22.

ETHNOPHARMACOLOGICAL RELEVANCE: Inonotus obliquus, also known as Chaga mushroom, is one of the most widely appreciated wild edible mushrooms in Russia and northern European countries and is renowned for its use in cancer treatment. Indeed, recently published in vitro and in vivo studies have demonstrated its anticancer activity in various types of cancer and support its potential application for therapeutic intervention in cancer. However, its activity against lung cancer, the most commonly diagnosed cancer and the leading cause of cancer death worldwide, and the underlying molecular basis of its action remain to be fully elucidated.

OBJECTIVE: This study aimed to evaluate the cytotoxic activity of I. obliquus in four human lung adenocarcinoma cell lines with different p53 status (A549, H1264, H1299, and Calu-6) and identify its active constituents by bioactivity-based analysis and the underlying molecular basis of their cytotoxicity on lung cancer cells.

MATERIALS AND METHODS: Bioactivity-guided fractionation and preparative/semi-preparative HPLC purification were used with LC/MS analysis to separate the bioactive constituents. Cell viability and apoptosis in human lung cancer cell lines (A549, H1264, H1299, and Calu-6) were assessed using the WST-1 assay and TUNEL staining, respectively. Caspase activation was assessed by detecting its surrogate markers, cleaved poly (ADP-ribose) polymerase (PARP) and caspase-3, using an immunoblot assay.

RESULTS: The MeOH extract of I. obliquus reduced cell viability in all lung cancer cell lines tested through induction of apoptosis accompanied by caspase-3 cleavage. Bioactivity-guided fractionation of the MeOH extract and chemical investigation of its cytotoxic hexane-soluble and CH2Cl2-soluble fractions led to the isolation of eight triterpenoids (1-8), including a new lanostane-type triterpenoid named chagabusone A (7). The structures of the isolates were elucidated based on spectroscopic analysis, including 1D and 2D NMR and high-resolution ESIMS. Among isolated compounds, compounds 1, 6, and 7 showed the most potent cytotoxic activity in all human lung cancer cell lines examined, with IC50 values ranging from 75.1 to 227.4 μM. Cytotoxicity of these compounds was mediated by apoptosis with caspase-3 activation.

CONCLUSION: These findings provide experimental evidence supporting the potential application of I. obliquus in lung cancer treatment and reveal the molecular basis underlying its cytotoxic activity against human lung cancer cells.

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Antiproliferative Activity and Cytotoxicity of Some Medicinal Wood-Destroying Mushrooms from Russia.

Int J Med Mushrooms. 2018; 20(1):1-11. doi: 10.1615/IntJMedMushrooms.2018025250.

We analyzed the antiproliferative activity of 6 medicinal wood-destroying mushrooms (Fomes fomentarius, Fomitopsis pinicola, Trametes versicolor, Trichaptum biforme, Inonotus obliquus, and Coniophora puteana) that are common in deciduous and mixed coniferous forests in Central Russia. Morphological identification of strains collected from the wild was confirmed based on ribosomal DNA internal transcribed spacer phylogenetic analysis. We observed cytotoxic and cell growth-inhibitory effects of hot water extracts from mycelial biomass of 5 species-T. versicolor, C. puteana, F. fomentarius, F. pinicola, and I. obliquus-on leukemia cell lines (Jukart, K562, and THP-1); the effective extract concentrations were mostly less than 50 µg · mL-1. However, we observed no antiproliferative activity of dry biomass from methanol-chloroform (1:1) extracts of C. puteana and F. fomentarius. A chemosensitivity assay showed that the most effective polypore mushroom extract was the methanol extract of T. versicolor (strain It-1), which inhibited the growth of 6 various solid tumors (A-549 and SWi573 [lung], HBL-100 and T-47D [breast], HeLa [cervix], and WiDr [colon]) at concentrations below 45 µg · mL-1, with a concentration as low as 0.7-3.6 µg · mL-1 causing 50% reduction in the proliferation of cancer cells in lung and cervix tumors. Methanol extracts of F. pinicola and I. obliquus were less effective, with proliferation-inhibiting capacities at concentrations below 70 and 200 µg · mL-1, respectively. Thus, T. versicolor is a prospective candidate in the search for and production of new antiproliferative chemical compounds.

Chaga (Inonotus obliquus), a Future Potential Medicinal Fungus in Oncology? A Chemical Study and a Comparison of the Cytotoxicity Against Human Lung Adenocarcinoma Cells (A549) and Human Bronchial Epithelial Cells (BEAS-2B).

Integr Cancer Ther. 2018 Sep; 17(3):832-843. doi: 10.1177/1534735418757912. Epub 2018 Feb 27.

BACKGROUND: Inonotus obliquus, also known as Chaga, is a parasitic fungus growing on birches and used in traditional medicine (especially by Khanty people) to treat various health problems. In this study, we aimed to quantify the 3 metabolites frequently cited in literature, that is, betulin, betulinic acid, and inotodiol in the Chaga recently discovered in forests located in Normandy (France), and to compare their concentrations with Ukrainian and Canadian Chaga. This study also explores the cytotoxicity of the French Chaga against cancer-derived cells and transformed cells.

METHODS: A quantification method by HPLC-MS-MS (high-performance liquid chromatography-tandem mass spectrometry) of betulin, betulinic acid, and inotodiol was developed to study the French Chaga and compare the concentration of these metabolites with extracts provided from Chaga growing in Canada and Ukraine. This method was also used to identify and quantify those 3 compounds in other traditional preparations of Chaga (aqueous extract, infusion, and decoction). Among these preparations, the aqueous extract that contains betulin, betulinic acid, and inotodiol was chosen to evaluate and compare its cytotoxic activity toward human lung adenocarcinoma cells (A549 line) and human bronchial epithelial cells (BEAS-2B line).

RESULTS: French Chaga contains betulin and betulinic acid at higher levels than in other Chaga, whereas the concentration of inotodiol is greater in the Canadian Chaga. Moreover, the results highlighted a cytotoxic activity of the Chaga's aqueous extract after 48 and 72 hours of exposure with a higher effect on cancer-derived cells A549 than on normal transformed cells BEAS-2B ($P = 0.025$ after 48 hours of exposure and $P = 0.004$ after 72 hours of exposure).

Inotodiol suppresses proliferation of breast cancer in rat model of type 2 diabetes mellitus via downregulation of β-catenin signaling.

Biomed Pharmacother. 2018 Mar; 99:142-150. doi: 10.1016/j.biopha.2017.12.084.

Breast cancer is amongst the most common cancers causing death of women worldwide. Breast cancer occurrence is more prominent in people with diabetes. A recent trend is management of diabetes and cancer has evolved to be natural remedy

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including single molecule therapy or combination. In this study, we investigated the effect of inotodiol on breast cancer growth in diabetic conditions. Inotodiol is a lanostane triterpenoid found in natural resources like edible mushroom Inonotus obliquus. We established a rat model of diabetic-breast cancer by treating female Sprague-Dawley rats with streptazotocin (STZ) at 35 mg/kg followed by induction of breast cancer by administration of 7,12-dimethylbenz(a)anthracene (DMBA) at 10 mg/kg. Diabetes development in experimental rats was confirmed by measuring fasting blood glucose levels and oral glucose tolerance test (OGTT), and other biochemical assays were performed. Histological evaluation of pancreas was performed. The proliferation of breast tumor was measured by immunohistochemical staining for PCNA, cleaved-caspase-3 and TUNEL staining for apoptosis, and β-catenin. Results of the study demonstrate that inotodiol lowered the blood glucose levels in SD rats as well as reduced plasma levels of cholesterol, triglyceride, and high-density lipoprotein. The tumor proliferation marker PCNA was reduced by inotodiol. It downregulated the expression of β-catenin and its downstream targets (c-Myc and Cyclin D1) followed by apoptosis induction. Conclusively, results suggest that inotodiol regulates blood glucose levels in diabetic rats and then controls proliferation of breast tumor progression by inducing apoptosis via downregulation of β-catenin signaling. It further suggests that inotodiol can be a preventive approach in managing dietary chronic conditions like diabetic-breast cancer.

Inonotus obliquus extract induces apoptosis in the human colorectal carcinoma's HCT-116 cell line.

Biomed Pharmacother. 2017 Dec; 96:1119-1126. doi: 10.1016/j.biopha.2017.11.111. Epub 2017 Nov 27.

Because of irregular dietary habits and lifestyle in Taiwan, the incidence and mortality rate of colorectal cancer have been increasing rapidly these years. This study investigated the inhibitory activity against the proliferation of human colorectal cancer HCT-116 cells by Inonotus obliquus extracts obtained from submerged fermentation. Cell viability was measured by the reduction of MTT and cell membrane integrity was determined by lactic dehydrogenase (LDH) release. The mRNA expression of proapoptosis and antiapoptosis mediators was assayed by real-time PCR, and the levels of p53 and NF-κB p65 were assessed using Western blot analysis. Furthermore, the influences of I. obliquus extracts to HCT-116 cells were evaluated by caspase-3 activity. The results can be summarized as, for the mitochondrial apoptotic pathway, quantitative RT-PCR data showed up-regulation of proapoptotic genes (Bax, bad, and caspase-3) and increased Bax/bcl-2 ratio by I. obliquus extracts. Moreover, treating with 20 mg/mL I. obliquus extracts augmented caspase-3 activity in HCT-116 cells. Induction of cell cycle G0/G1 phase arrest: I. obliquus extracts up-regulated the mRNA expression of proapoptotic genes (p53, p21WAF1/CIP1) and down-regulated antiapoptotic gene (CyclinD1), while extracts of I. obliquus mycelia increased the expressions of p53 protein in HCT-116 cells. I. obliquus extracts decreased the expression of NF-κB p65 protein and COX-2 gene in HCT-116 cells. Taking together, I. obliquus extracts may be used as a potentially novel food material for health care to improve the treatment of colorectal cancer.

Polysaccharide isolated from the liquid culture broth of Inonotus obliquus suppresses invasion of B16-F10 melanoma cells via AKT/NF-κB signaling pathway.

Mol Med Rep. 2016 Nov;14(5):4429-4435. doi: 10.3892/mmr.2016.5771. Epub 2016 Sep 23.

A number of polysaccharides exhibit pharmacological activities. Polysaccharides derived from Inonotus obliquus (PLIO) appear to have various potential pharmacological properties, including anti-tumor activity. However, the molecular mechanisms underlying these properties remain to be elucidated. The present study investigated the anti-metastatic potential of PLIO and the underlying signaling pathways in B16-F10 murine melanoma cells using the MTT colorimetric assay, in vitro migration and invasion assays, and flow cytometric and western blot analyses. PLIO inhibited the invasion of B16-F10 cells and suppressed the expression of matrix metalloproteinases. PLIO treatment inhibited nuclear factor-κB (NF-κB) nuclear translocation in B16-F10 cells. In addition, PLIO treatment inhibited the phosphorylation of c-Jun N-terminal kinases and AKT. These results suggest that PLIO may suppress the invasion of highly metastatic melanoma cells via inhibition of the AKT/NF-κB signaling pathways.

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Continuous intake of the Chaga mushroom (Inonotus obliquus) aqueous extract suppresses cancer progression and maintains body temperature in mice.

Heliyon. 2016 May 12;2(5):e00111. doi: 10.1016/j.heliyon.2016.e00111. eCollection 2016 May.

AIMS: Cancer is a leading cause of morbidity and mortality worldwide; therefore, effective measures for cancer prevention and treatment are in constant demand. The extracts of Inonotus obliquus (Chaga mushroom) demonstrate potent anti-tumor activities and have been used to treat cancer in several countries; however, the actual effect and underlying mechanisms are still unclear. In the present study, we aimed to investigate the effects of continuous intake of aqueous extract from I. obliquus on tumor suppression.

MAIN METHODS: Anticancer activity of the I. obliquus extract was examined in mouse models of Lewis lung carcinoma growth and spontaneous metastasis after 3 weeks of continuous extract intake at the dose of 6 mg/kg/day, which corresponded to that ingested daily with Chaga infusion in Japan.

KEY FINDINGS: The extract of I. obliquus caused significant tumor suppressive effects in both models. Thus, in tumor-bearing mice, 60% tumor reduction was observed, while in metastatic mice, the number of nodules decreased by 25% compared to the control group. Moreover, I. obliquus extract-treated mice demonstrated the increase in tumor agglomeration and inhibition of vascularization. Interestingly, I. obliquus intake decreased body weight in middle-aged mice and increased body temperature in response to light-dark switching in mature adult mice. Furthermore, I. obliquus prevented temperature drop in mice after tumor implantation.

SIGNIFICANCE: Our findings suggest that the I. obliquus extract could be used as a natural remedy for cancer suppression by promoting energy metabolism.

Chemical constituents from Inonotus obliquus and their antitumor activities.

J Nat Med. 2016 Oct;70(4):721-30. doi: 10.1007/s11418-016-1002-4. Epub 2016 May 14.

Four new lanostane-type triterpenes (inonotusanes D-G, 1-4), including a 24,25,26,27-tetranorlanostane, together with 11 known compounds (5-15), including 7 lanostane derivatives, 2 steroids and 2 aromatic compounds, were isolated from the sclerotia of Inonotus obliquus. Their structures were elucidated by 1D and 2D NMR spectroscopy and HRMS. To our knowledge, 1 is the first 24,25,26,27-tetranorlanostane-type triterpenoid from fungus, and this is the first time that 31-member lanostane-type triterpenes, 5 and 6, have been isolated from the sclerotia of I. obliquus instead of from its submerged culture. 7 and 8 are also new isolates of this genus. Compounds 1, 8, 12 and 13 exhibited strong cytotoxicity against the 4T1 (mouse breast cancer) cell line, with IC50 9.40, 7.79, 9.06 and 9.31 μM, respectively. 8, 12 and 13 also exhibited strong cytotoxicity against the the MCF-7 (human breast cancer) cell line, with IC50 8.35-9.01 μM.

Ergosterol peroxide from Chaga mushroom (Inonotus obliquus) exhibits anti-Cancer activity by down-regulation of the β-catenin pathway in colorectal Cancer.

J Ethnopharmacol. 2015 Jul 22. pii: S0378-8741(15)30047-7. doi: 10.1016/j.jep.2015.07.030. [Epub ahead of print]

AIM OF THE STUDY: In this study, we examined the effect of different fractions and components of Chaga mushroom (Inonotus Obliquus) on viability and apoptosis of colon cancer cells. Among them, one component showed the most effective growth inhibition and was identified as ergosterol peroxide by NMR analysis. We investigated the anti-proliferative and apoptosis mechanisms of ergosterol peroxide associated with its anti-cancer activities in human colorectal cancer (CRC) cell lines and tested its anti-tumor effect on colitis-induced CRC developed by Azoxymethane (AOM)/Dextran sulfate sodium (DSS) in a mouse model.

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MATERIALS AND METHODS: We used MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assays, flow cytometry assays, Western blot analysis, colony formation assays, reverse transcription-polymerase chain reaction (RT-PCR), immunohistochemistry (IHC), and AOM/DSS mouse models to study the molecular mechanism of metastatic activities in CRC cells.

RESULTS: Ergosterol peroxide inhibited cell proliferation and also suppressed clonogenic colony formation in HCT116, HT-29, SW620 and DLD-1 CRC cell lines. The growth inhibition observed in these CRC cell lines was the result of apoptosis, which was confirmed by FACS analysis and Western blotting. Ergosterol peroxide inhibited the nuclear levels of β-catenin, which ultimately resulted in reduced transcription of c-Myc, cyclin D1, and CDK-8. Ergosterol peroxide administration showed a tendency to suppress tumor growth in the colon of AOM/DSS-treated mice, and quantification of the IHC staining showed a dramatic decrease in the Ki67-positive staining and an increase in the TUNEL staining of colonic epithelial cells in AOM/DSS-treated mice by ergosterol peroxide for both prevention and therapy.

CONCLUSION: Our data suggest that ergosterol peroxide suppresses the proliferation of CRC cell lines and effectively inhibits colitis-associated colon cancer in AOM/DSS-treated mice. Ergosterol peroxide down-regulated β-catenin signaling, which exerted anti-proliferative and pro-apoptotic activities in CRC cells. These properties of ergosterol peroxide advocate its use as a supplement in colon cancer chemoprevention.

Purification, characterization and biological activity of a novel polysaccharide from Inonotus obliquus.

Int J Biol Macromol. 2015 Aug; 79:587-94. doi: 10.1016/j.ijbiomac.2015.05.016. Epub 2015 May 27.

A novel water-soluble polysaccharide IP3a was successfully isolated and purified from I. obliquus by DEAE-cellulose, Sepharose CL-6B and Sephadex G-200 column chromatography. Chemical characterization and antitumor and immunoregulatory activity of IP3a were investigated. IP3a consisted of rhamnose, arabinose, glucose and galactose in a molar ratio of 2.5:4.6:1.0:2.6 with an average molecular weight of 48,820 Da. IP3a exhibited no significant antitumor activities in vitro. However, IP3a could not only inhibit the growth of transplantable Jurkat tumor in mice significantly, but also could enhance the splenocyte proliferation and lymphocyte proliferation induced by ConA and LPS in a dose-dependent manner. At the same time, IP3a could promote cytokine secretion (IL-2, IL-6, IL-12 and TNF-α) and macrophage phagocytosis in mice. In addition, IP3a could increase Bax expression and inhibit Bcl-2 expression significantly. These results suggested that antitumor mechanisms of IP3a might be associated with improving immune response in vivo and inducing apoptosis of tumor cells in vitro. IP3a might be utilized as a potential therapeutic agent against lymphoma cancer with immunomodulatory activity.

Ethanol extract of Innotus obliquus (Chaga mushroom) induces G1 cell cycle arrest in HT-29 human colon cancer cells.

Nutr Res Pract. 2015 Apr; 9(2):111-6. doi: 10.4162/nrp.2015.9.2.111. Epub 2015 Mar 12.

BACKGROUND/OBJECTIVES: Inonotus obliquus (I. obliquus, Chaga mushroom) has long been used as a folk medicine to treat cancer. In the present study, we examined whether or not ethanol extract of I. obliquus (EEIO) inhibits cell cycle progression in HT-29 human colon cancer cells, in addition to its mechanism of action.

MATERIALS/METHODS: To examine the effects of Inonotus obliquus on the cell cycle progression and the molecular mechanism in colon cancer cells, HT-29 human colon cancer cells were cultured in the presence of 2.5 - 10 μg/mL of EEIO, and analyzed the cell cycle arrest by flow cytometry and the cell cycle controlling protein expression by Western blotting.

RESULTS: Treatment cells with 2.5 - 10 μg/mL of EEIO reduced viable HT-29 cell numbers and DNA synthesis, increased the percentage of cells in G1 phase, decreased protein expression of CDK2, CDK4, and cyclin D1, increased expression of p21, p27, and p53, and inhibited phosphorylation of Rb and E2F1 expression. Among I. obliquus fractions, fraction 2 (fractionated by dichloromethane from EEIO) showed the same effect as EEIO treatment on cell proliferation and cell cycle-related protein levels.

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CONCLUSIONS: These results demonstrate that fraction 2 is the major fraction that induces G1 arrest and inhibits cell proliferation, suggesting I. obliquus could be used as a natural anti-cancer ingredient in the food and/or pharmaceutical industry.

Triterpenoids from Inonotus obliquus and their antitumor activities.

Fitoterapia. 2015 Mar; 101:34-40. doi: 10.1016/j.fitote.2014.12.005. Epub 2014 Dec 24.

Three new lanostane-type triterpenes, inonotusanes A-C (1-3), and a new naturally occurring one, 3β-hydroxy-25,26,27-trinorlanosta-8,22E-dien-24-oic acid (4), together with sixteen known triterpenoids (5-20), including 13 lanostane derivatives, 2 lupanes and 1 oleanane-type triterpene were isolated from the sclerotia of Inonotus obliquus. Their structures were elucidated by 1D and 2D NMR spectroscopy and HRMS. Compounds 6, 8, 18 and 20 exhibited strong cytotoxicity against A549 tumor cell lines, with IC50 values of 2.34, 1.63, 8.39 and 5.39μM, respectively. Seven compounds (3, 9, 10, 12, 18-20) exhibited moderate cytotoxicity against A549, HT29, Hela or L1210 tumor cell lines.

Inonotus obliquus-derived polysaccharide inhibits the migration and invasion of human non-small cell lung carcinoma cells via suppression of MMP-2 and MMP-9.

Int J Oncol. 2014 Dec;45(6):2533-40. doi: 10.3892/ijo.2014.2685. Epub 2014 Sep 30.

Polysaccharides isolated from the fruiting body of Inonotus obliquus (PFIO) are known to possess various pharmacological properties including antitumor activity. However, the anti-metastatic effect and its underlying mechanistic signaling pathway involved these polysaccharides in human non-small cell lung carcinoma remain unknown. The present study therefore aimed to determine the anti-metastatic potential and signaling pathways of PFIO in the highly metastatic A549 cells. We found that PFIO suppressed the migration and invasive ability of A549 cells while decreasing the expression levels and activity of matrix metalloproteinase (MMP)-2 and MMP-9. Furthermore, PFIO decreased the phosphorylation levels of mitogen-activated protein kinases (MAPKs) and phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT) as well as the expression level of COX-2, and inhibited the nuclear translocation of nuclear factor κB (NF-κB) in A549 cells. These results suggested that PFIO could suppress the invasion and migration of human lung carcinoma by reducing the expression levels and activity of MMP-2 and MMP-9 via suppression of MAPKs, PI3K/AKT, and NF-κB signaling pathways.

CARI III inhibits tumor growth in a melanoma-bearing mouse model through induction of G0/G1 cell cycle arrest.

Molecules. 2014 Sep 12; 19(9):14383-95. doi: 10.3390/molecules190914383.

Mushroom-derived natural products have been used to prevent or treat cancer for millennia. In this study, we evaluated the anticancer effects of CARI (Cell Activation Research Institute) III, which consists of a blend of mushroom mycelia from Phellinus linteus grown on germinated brown rice, Inonotus obliquus grown on germinated brown rice, Antrodia camphorata grown on germinated brown rice and Ganoderma lucidum. Here, we showed that CARI III exerted anti-cancer activity, which is comparable to Dox against melanoma in vivo. B16F10 cells were intraperitoneally injected into C57BL6 mice to develop solid intra-abdominal tumors. Three hundred milligrams of the CARI III/kg/day p.o. regimen reduced tumor weight, comparable to the doxorubicin (Dox)-treated group. An increase in life span (ILS% = 50.88%) was observed in the CARI III-administered group, compared to the tumor control group. CARI III demonstrates anti-proliferative activity against B16F10 melanoma cells through inducing G0/G1 cell cycle arrest. CARI III inhibits the expression of cyclin D1, CDK4 and CDK2 and induces p21. Therefore, CARI III could be a potential chemopreventive supplement to melanoma patients.

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Inhibitory effects of a polysaccharide extract from the Chaga medicinal mushroom, Inonotus obliquus (higher Basidiomycetes), on the proliferation of human neurogliocytoma cells.

Int J Med Mushrooms. 2014; 16(1):29-36.

This study aimed to investigate the inhibitory roles of a polysaccharide extract from Inonotus obliquus on U251 human neurogliocytoma cells cultured in vitro. After administering the polysaccharide extract from I. obliquus to U251 cells cultivated in vitro, methyl thiazolyl tetrazoliym assay was performed to measure the inhibitory effects of the extract on tumor cell proliferation. The expression of the apoptosis-related proteins Bcl-2 and caspase-3 were determined by Western blotting. Different concentrations of I. obliquus extract (25, 50, 100, 200, and 500 μg/mL) were added to U251 cells at 24, 48, and 72 hours. Methyl thiazolyl tetrazoliym assay showed that the inhibition ratio increased with increased extract concentration and prolonged treatment duration. The I. obliquus extract sharply decreased the expression of Bcl-2 but dramatically increased the expression of caspase-3. This function was gradually enhanced with increased drug concentration and prolonged treatment duration. The I. obliquus extract can inhibit the proliferation of tumor cells. This inhibition function is closely related to the downregulation of Bcl-2 and the upregulation of caspase-3.

Inotodiol inhabits proliferation and induces apoptosis through modulating expression of cyclinE, p27, bcl-2, and bax in human cervical cancer HeLa cells.

Asian Pac J Cancer Prev. 2014; 15(7):3195-9.

Inonotus obliquus is a medicinal mushroom that has been used as an effective agent to treat various diseases such as diabetes, tuberculosis and cancer. Inotodiol, an included triterpenoid shows significant anti-tumor effect. However, the mechanisms have not been well documented. In this study, we aimed to explore the effect of inotodiol on proliferation and apoptosis in human cervical cancer HeLa cells and investigated the underlying molecular mechanisms. HeLa cells were treated with different concentrations of inotodiol. The MTT assay was used to evaluate cell proliferating ability, flow cytometry (FCM) was employed for cell cycle analysis and cell apoptosis, while expression of cyclinE, p27, bcl-2 and bax was detected by immunocytochemistry. Proliferation of HeLa cells was inhibited by inotodiolin a dose-dependent manner at 24h ($r=0.9999$, $p<0.01$). A sub-G1 peak (apoptotic cells) of HeLa cells was detected after treatment and the apoptosis rate with the concentration and longer incubation time ($r=1.0$, $p<0.01$), while the percentage of cells in S phase and G2/M phase decreased significantly. Immunocytochemistry assay showed that the expression of cyclin E and bcl-2 in the treated cells significantly decreased, while the expression of p27 and bax obviously increased, compared with the control group ($p<0.05$). The results of our research indicate that inotodiol isolated from Inonotus obliquus inhibited the proliferation of HeLa cells and induced apoptosis in vitro. The mechanisms may be related to promoting apoptosis through increasing the expression of bax and cutting bcl-2 and affecting the cell cycle by down-regulation the expression of cyclin E and up-regulation of p27. The results further indicate the potential value of inotodiol for treatment of human cervical cancer.

Polysaccharide from Inonotus obliquus inhibits migration and invasion in B16-F10 cells by suppressing MMP-2 and MMP-9 via downregulation of NF-κB signaling pathway.

Oncol Rep. 2014 May; 31(5):2447-53. doi: 10.3892/or.2014.3103. Epub 2014 Mar 21.

Polysaccharides derived from Inonotus obliquus (PIO) are known to possess multiple pharmacological activities including antitumor activity. However, the possible molecular mechanisms of these activities are unknown. In the present study, we determined the anti-metastatic potential and signaling pathways of PIO in the highly metastatic B16-F10 mouse melanoma cell line in vitro. We found that PIO suppressed the migration and invasive ability of B16-F10 cells and decreased the expression levels and activities of matrix metalloproteinase (MMP)-2 and MMP-9. In addition, PIO decreased the phosphorylation levels of extracellular signal-regulated protein kinase (ERK), c-Jun N-terminal kinase (JNK) and p38 mitogen-activated protein kinase

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(MAPK); PIO also decreased the expression level of cyclooxygenase (COX) -2 and inhibited the nuclear translocation of nuclear factor κB (NF-κB) in B16-F10 melanoma cells. These results suggest that PIO could suppress the invasion and migration of B16-F10 melanoma cells by reducing the expression levels and activities of MMP-2 and MMP-9 through suppressing MAPK, COX-2 and NF-κB signaling pathways.

Chemical constituents from Inonotus obliquus and their biological activities.

J Nat Prod. 2014 Jan 24; 77(1):35-41. doi: 10.1021/np400552w. Epub 2013 Dec 20.

Seven new triterpenes, inonotusol A-G (1-7), one new diterpene, inonotusic acid (8), and 22 known compounds were isolated from Inonotus obliquus. Their structures were elucidated on the basis of spectroscopic analysis, including homonuclear and heteronuclear correlation NMR ((1)H-(1)H COSY, ROESY, HSQC, and HMBC) experiments. In in vitro assays, compounds 6 and 8-16 showed hepatoprotective effects against d-galactosamine-induced WB-F344 cell damage, with inhibitory effects from 34.4% to 81.2%. Compounds 7, 17, and 18 exhibited selective cytotoxicities against KB, Bel-7402, or A-549 cell lines. Compounds 16 and 17 showed inhibitory effects against protein tyrosine kinases, with IC50 values of 24.6 and 7.7 μM, respectively.

Inhibitory effects of low molecular weight polyphenolics from Inonotus obliquus on human DNA topoisomerase activity and cancer cell proliferation.

Mol Med Rep. 2013 Aug; 8(2):535-42. doi: 10.3892/mmr.2013.1547. Epub 2013 Jun 25.

Low molecular weight (LMW) polyphenolics containing a polyhydroxylated benzyl moiety are abundant in medicinal plants. In the present study, we report on the activities of seven LMW polyphenolics isolated from Inonotus obliquus, a medicinal mushroom. The isolated compounds included caffeic acid (CA), 3,4-dihydroxybenzalacetone (DBL), gallic acid, syringic acid, protocatechuic acid, 3,4-dihydroxybenzaldehyde and 2,5-dihydroxyterephthalic acid. We analyzed their inhibitory effects on DNA polymerase (pol) and DNA topoisomerase (topo), and their effects on human cancer cell growth. All isolated compounds inhibited human topo II activity; the most potent were DBL and CA, which contain a catechol propanoid moiety. CA and DBL inhibited the activity of human topo I, whereas other compounds had no effect. No compound modulated the activities of 11 mammalian pol species or other DNA metabolic enzymes, including T7 RNA polymerase, mouse IMP dehydrogenase (type II), T4 polynucleotide kinase and bovine deoxyribonuclease I. CA and DBL markedly suppressed the proliferation of human colon HCT116 carcinoma cells with an LD50 of 70.0 and 49.4 μM, respectively, and halted the cell cycle in the G2/M phase. The suppressive effect of these compounds on cancer cell growth correlated with their ability to inhibit topo II. These results suggest that CA- and DBL-dependent decreases in cell proliferation are due to the inhibition of cellular topo II. The mechanism of action of these catechol propanoid compounds and the implication for their use as anticancer agents are discussed.

Progress on understanding the anticancer mechanisms of medicinal mushroom: inonotus obliquus.

Asian Pac J Cancer Prev. 2013; 14(3):1571-8.

Cancer is a leading cause of death worldwide. Recently, the demand for more effective and safer therapeutic agents for the chemoprevention of human cancer has increased. As a white rot fungus, Inonotus obliquus is valued as an edible and medicinal resource. Chemical investigations have shown that I. obliquus produces a diverse range of secondary metabolites, including phenolic compounds, melanins, and lanostane-type triterpenoids. Among these are active components for antioxidant, antitumoral, and antiviral activities and for improving human immunity against infection of pathogenic microbes. Importantly, their anticancer activities have become a hot recently, but with relatively little knowledge of their modes of action. Some compounds extracted from I. obliquus arrest cancer cells in the G0/G1 phase and then induce cell apoptosis or differentiation, whereas some examples directly participate in the cell apoptosis pathway. In other cases, polysaccharides from I. obliquus can indirectly be involved in anticancer processes mainly via stimulating the immune system. Furthermore, the antioxidative ability

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of I. obliquus extracts can prevent generation of cancer cells. In this review, we highlight recent findings regarding mechanisms underlying the anticancer influence of I. obliquus, to provide a comprehensive landscape view of the actions of this mushroom in preventing cancer.

Anticancer effects of fraction isolated from fruiting bodies of Chaga medicinal mushroom, Inonotus obliquus (Pers.:Fr.) Pilát (Aphyllophoromycetideae): in vitro studies.

Int J Med Mushrooms. 2011; 13(2):131-43.

The medicinal mushroom Chaga, Inonotus obliquus (Pers.:Fr.) Pilát (Hymenochaetaceae), has been used in folk medicine in Russia, Poland, and most of the Baltic countries, as a cleansing and disinfecting measure, and as decoctions for stomach diseases, intestinal worms, liver and heart ailments, and cancer treatment. Many reports have been published concerning the health promoting functions of this mushroom, including antibacterial, hepatoprotective, anti-inflammatory, antitumor, and antioxidant activities. The purpose of the present study was evaluation of in vitro anticancer activity of fraction IO4 isolated from I. obliquus. The effect on cell proliferation, motility and viability was assessed in a range of cancer and normal cells. Chaga fraction prepared from dried fruiting bodies was subjected to anticancer evaluation in human lung carcinoma (A549), colon adenocarcinoma (HT-29), and rat glioma (C6) cell cultures. Human skin fibroblasts (HSF), bovine aorta endothelial cells (BAEC), models of rat oligodendrocytes (OLN-93), hepatocytes (Fao), rat astroglia, and mouse neurons (P19) were applied to test toxicity in normal cells. The following methods were applied: tumor cell proliferation (MTT assay and BrdU assay), cytotoxicity (LDH assay), tumor cell motility (wound assay), tumor cell morphology (May-Grünwald-Giemsa staining), and death detection (ELISA). Chaga fraction elicited anticancer effects which were attributed to decreased tumor cell proliferation, motility and morphological changes induction. Of note is the fact that it produced no or low toxicity in tested normal cells. The data presented could open interesting paths for further investigations of fraction IO4 as a potential anticancer agent.

In vitro antitumor activity and structure characterization of ethanol extracts from wild and cultivated Chaga medicinal mushroom, Inonotus obliquus (Pers.:Fr.) Pilát (Aphyllophoromycetideae).

Int J Med Mushrooms. 2011; 13(2):121-30.

Inonotus obliquus (Pers.:Fr.) Pilát has been traditionally used as a folk remedy for treatment of cancers, cardiovascular disease and diabetes in Russia, Poland, and most of the Baltic countries, but natural reserves of this fungus have nearly been exhausted. This study was designed to investigate the artificial cultivation of I. obliquus and the antitumor activity of its tissues. The ethanol extract of cultivated sclerotium had the highest cell growth inhibitory rate (74.6%) as determined by an 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. 78% of the bags produced sclerotia and only 6.17 g/bag of sclerotium was obtained. Extracts of the cultivated fruiting body showed 44.2% inhibitory activity against tumor cells. However, the yield was as high as 18.24 g/bag, and 98% of the bags produced fruiting body. The results of gas chromatography-mass spectroscopy (GC-MS) showed that similar compounds were extracted from the wild and cultivated samples. The principal compounds observed were lanosterol, inotodiol, and ergosterol. Their percentages of the mass fraction were 86.1, 59.9, and 71.8% of the total, for the wild sclerotium, cultivated sclerotium, and cultivated fruiting body, respectively. Ergosterol was found to be much higher (27.32%) in cultivated fruiting body. We conclude that cultivated fruiting body of I. obliquus obtained by inoculation of the substrate with spawn mycelium of the fifth generation could serve as an ideal substitute for the wild I. obliquus.

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Effects of inotodiol extracts from inonotus obliquus on proliferation cycle and apoptotic gene of human lung adenocarcinoma cell line A549.

Chin J Integr Med. 2011 Mar; 17(3):218-23. doi: 10.1007/s11655-011-0670-x. Epub 2011 Feb 27.

OBJECTIVE: To observe the proliferation inhibition, apoptosis, and cell proliferation cycle of human lung carcinoma cell line A549 treated with Inotodiol extracts from Inonotus obliquus and explore the possibility of Inotodiol extracts from Inonotus obliquus as a new tumor chemopreventive drug.

METHODS: Human lung cancer cell line A549 was treated with different concentrations of Inotodiol, the effects of Inotodiol on cell apoptosis, the expression of Ki-67, Bcl-2, Bax, and p53 and cell cycle were detected by TUNEL assay, immunohistochemistry, and flow cytometry assay respectively.

RESULTS: Inotodiol extracts had antiproliferation effect on human lung carcinoma cell line A549. The expression of Ki-67 decreased with the increase of Inotodiol concentration and exposure time (P<0.05), in a dose-dependent and time-dependent manner. The typical characteristics of the apoptosis of A549 cells treated with Inotodiol were observed, and the apoptotic rate of A549 cell at 48 h was the highest by TUNEL assay. Inotodiol arrested A549 cells in the S phase, and apoptotic peak was observed by flow cytometry. Immunocytochemistry indicated that the expression of Bcl-2 protein decreased, while the expression of p53 and Bax proteins increased in A549 cells treated with Inotodiol, compared with the control cells (P<0.05).

CONCLUSION: Inotodiol can inhibit proliferation and induce the apoptosis of A549 cells, and its molecular mechanism may be associated with the up-regulating expression of p53 and bax proteins and down-regulating expression of Bcl-2 protein, which arrested A549 cells in S phase.

Separation of an aqueous extract Inonotus obliquus (Chaga). A novel look at the efficiency of its influence on proliferation of A549 human lung carcinoma cells.

Acta Pol Pharm. 2010 Jul-Aug; 67(4):397-406.

Aqueous extract of Inonotus obliquus was hydrolyzed in dilute hydrochloric acid. The products were extracted applying organic solvents and separated chromatographically on a silica gel-packed column. Eluted fractions were analyzed by means of GC-MS. The presence of hydrocarbons, alcohols, phenols and various carbonyl compounds in analyzed fractions has been detected and quantified. Preliminarily experiments on the influence of certain separated samples on the proliferation of A549 human lung carcinoma cells were performed. Therefore, we hypothesize that the major antiproliferative effects are related to the presence of benzaldehyde, which is a benzyl alcohol metabolite formed in situ in the cells culture with the yield moderated by the presence of trace amounts of "high molecular mass compounds".

Anticancer activity of subfractions containing pure compounds of Chaga mushroom (Inonotus obliquus) extract in human cancer cells and in Balbc/c mice bearing Sarcoma-180 cells.

Nutr Res Pract. 2010 Jun; 4(3):177-82. doi: 10.4162/nrp.2010.4.3.177. Epub 2010 Jun 29.

The Chaga mushroom (Inonotus obliquus) has been used in folk medicine to treat cancers. However, limited information exists on the underlying anticancer effects of the major component of I. obliquusin vivo. We hypothesize that the pure compounds (3beta-hydroxy-lanosta-8,24-dien-21-al, inotodiol and lanosterol, respectively) separated from I. obliquus would inhibit tumor growth in Balbc/c mice bearing Sarcoma-180 cells (S-180) in vivo and growth of human carcinoma cells in vitro. To test this hypothesis, the growth inhibition of each subfraction isolated from I. obliquus on human carcinoma cell lines (lung carcinoma A-549 cells, stomach adenocarcinoma AGS cells, breast adenocarcinoma MCF-7 cells, and cervical adenocarcinoma HeLa cells) was tested in vitro. Then, after S-180 implantation, the mice were fed a normal chow supplemented with 0, 0.1 or 0.2 mg of subfraction 1, 2 or 3 per mouse per day. All of the subfractions isolated from I. obliquus showed significant cytotoxic activity

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against the selected cancer cell lines in vitro. Subfraction 1 was more active than subfraction 2 and subfraction 3 against the A549, AGS and MCF-7 cancer cell lines in vitro. In in vivo results, subfraction 1 isolated from I. obliquus at concentrations of 0.1 and 0.2 mg/mouse per day significantly decreased tumor volume by 23.96% and 33.71%, respectively, as compared with the control. Subfractions 2 and 3 also significantly inhibited tumor growth in mice bearing S-180 as compared with the control mouse tumor. Subfraction 1 isolated from I. obliquus showed greater inhibition of tumor growth than subfractions 2 and 3, which agrees well with the in vitro results. The results suggest that I. obliquus and its compounds in these subfractions isolated from I. obliquus could be used as natural anticancer ingredients in the food and/or pharmaceutical industry.

Antitumor activity of water extract of a mushroom, Inonotus obliquus, against HT-29 human colon cancer cells.

Phytother Res. 2009 Dec; 23(12):1784-9. doi: 10.1002/ptr.2836.

In the current study, it was demonstrated that the hot water extract of I. obliquus (IOWE) exerts inhibitory activity against the proliferation of human colon cancer cells (HT-29). The inhibitory effect of IOWE on the growth of HT-29 cancer cells was evaluated by treating cells with IOWE at concentrations of 0.25, 0.5 and 1.0 mg/mL for 24 or 48 h. The IOWE inhibited cell growth in a dose-dependent manner, and this inhibition was accompanied by apoptotic cell death. The maximum inhibitory effect (56%) was observed when IOWE was treated at a concentration of 1.0 mg/mL for 48 h. The apoptotic effect of IOWE on HT-29 cells was also confirmed by flow cytometric analysis. In addition, the apoptotic cell percentage was closely associated with down-regulation of Bcl-2 and up-regulation of Bax and caspase-3. The results suggest that IOWE would be useful as an antitumor agent via the induction of apoptosis and inhibition of the growth of cancer cells through up-regulation of the expression of proapoptotic proteins and down-regulation of antiapoptotic proteins.

Cytostatic activity of peptide extracts of medicinal plants on transformed A549, H1299, and HeLa Cells.

Bull Exp Biol Med. 2009 Jan; 147(1):48-51.

Biological activity of peptide extracts of medicinal plants was studied on transformed non-small-cell lung carcinoma A549 cells, lung cancer H1299 cells, and cervical cancer HeLa cells at various cell densities. Cell survival and proliferation were evaluated 72 h after treatment with extracts in concentrations of 0.05, 0.25, and 0.5 microg/microl. The cytostatic effect was produced by peptide extracts of Camelia sinesis Kuntze, Inonotus obliquus, and a mixture Inula helenium L., Chelidonium majus L., Equisetum arvense L., and Inonotus obliquus. Peptide extracts of Hypericum perforatum L. and Laurus nobilis L. in the same concentrations had no effects on proliferative activity and growth of tumor cells.

Cancer cell cytotoxicity of extracts and small phenolic compounds from Chaga [Inonotus obliquus (persoon) Pilat].

J Med Food. 2009 Jun; 12(3):501-7. doi: 10.1089/jmf.2008.1149.

Previously, we studied the antioxidant potential of Chaga mushroom [Inonotus obliquus (persoon) Pilat] extracts and isolated several small (poly)phenolic compounds as the major antioxidant components in the 80% methanol (MeOH) extract. In the present study, these isolated phenolic ingredients together with several other types of Chaga extracts were examined for cytotoxic effects against normal (IMR90) and cancer (A549, PA-1, U937, and HL-60) cell lines. Results revealed decoctions from both the fruiting body (FB) and sclerotium (ST) parts of Chaga, especially the ST part, showed considerable cytotoxicity toward tumor cells, but the cytotoxicity appeared to be stronger against normal cells than cancer cells. The 80% MeOH ST extract also showed the same trend. On the other hand, the 80% MeOH extract of FB showed significant cytotoxicity towards tumor cell lines without affecting normal cells, for example, the 50% lethal dose was 49.4 +/- 2.9 microg/mL for PA-1 cells versus 123.6 +/- 13.8 microg/mL for normal cells. The phenolic components isolated from the 80% MeOH extracts had markedly greater cancer

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cell toxicity than the extracts themselves. In particular, two out of seven compounds showed strong cytotoxicity towards several tumor cell lines without giving rise to significant cell toxicity toward normal cells. For example, the 50% lethal dose for 3,4-dihydroxybenzalacetone was 12.2 micromol/L in PA-1 cells but was 272.8 micromol/L in IMR90 cells. Fluorescence-activated cell sorting analysis further revealed these phenolic ingredients have high potentiality for apoptosis induction in PA-1 cells.

Evaluation of antitumor activity of peptide extracts from medicinal plants on the model of transplanted breast cancer in CBRB-Rb(8.17)Iem mice.

Bull Exp Biol Med. 2008 Apr; 145(4):464-6.

We studied antitumor effects of peptide extracts from plants on slowly growing mammary adenocarcinoma in CBRB-Rb(8.17)1Iem mice used as a model of breast cancer in humans. The antitumor effect of a single injection of the test peptides was evaluated by the delay of the appearance and growth of palpable breast cancer in mice over 4 weeks. Peptides from Hypericum perforatum and a mixture of Chelidonium majus L., Inula helenium L., Equisetum arvense L., and Inonotus obliquus exhibited maximum activity. Peptide extracts from Frangula alnuc Mill. and Laurus nobilis L. were less active. No antitumor effect of Camelia sinesis Kuntze was detected.

Potential anticancer properties of the water extract of Inonotus [corrected] obliquus by induction of apoptosis in melanoma B16-F10 cells.

J Ethnopharmacol. 2009 Jan 21; 121(2):221-8. doi: 10.1016/j.jep.2008.10.016. Epub 2008 Oct 25.

ETHNOPHARMACOLOGICAL RELEVANCE: Inonotus obliquus (Chaga mushroom), one of the widely known medicinal mushrooms, has been used to treat various cancers in Russia and most of Baltic countries for many centuries.

AIM OF THE STUDY: To examine the anti-proliferative effects of Inonotus obliquus extract on melanoma B16-F10 cells. Furthermore, to assess the anti-tumor effect of Inonotus obliquus extract in vivo in Balb/c mice.

MATERIALS AND METHODS: The water extract of Inonotus obliquus was studied for anti-proliferative effects on the growth and morphology of B16-F10 melanoma cells and for anti-tumor effect using in vivo in Balb/c mice.

RESULTS: Inonotus obliquus extract not only inhibited the growth of B16-F10 cells by causing cell cycle arrest at G(0)/G(1) phase and apoptosis, but also induced cell differentiation. These effects were associated with the down-regulation of pRb, p53 and p27 expression levels, and further showed that Inonotus obliquus extract resulted in a G(0)/G(1) cell cycle arrest with reduction of cyclin E/D1 and Cdk 2/4 expression levels. Furthermore, the anti-tumor effect of Inonotus obliquus extract was assessed in vivo in Balb/c mice. Intraperitoneal administration of Inonotus obliquus extract significantly inhibited the growth of tumor mass in B16-F10 cells implanted mice, resulting in a 3-fold (relative to the positive control, (*)p<0.05) inhibit at dose of 20mg/kg/day for 10 days.

CONCLUSION: This study showed that the water extract of Inonotus obliquus mushroom exhibited a potential anticancer activity against B16-F10 melanoma cells in vitro and in vivo through the inhibition of proliferation and induction of differentiation and apoptosis of cancer cells.

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Inotodiol, a lanostane triterpenoid, from Inonotus obliquus inhibits cell proliferation through caspase-3-dependent apoptosis.

Anticancer Res. 2008 Sep-Oct; 28(5A):2691-6.

BACKGROUND: To investigate the antitumor effect of Inonotus obliquus Pilat, the antiproliferative effect of lanostane triterpenoids from a chloroform extract of I. obliquus sclerotia against mouse leukemia P388 cells was assessed.

MATERIALS AND METHODS: Cell viability was measured by MTT assay. Caspase-3/7 activity and DNA fragmentation were evaluated to analyze apoptosis induction. The in vivo antitumor effect was evaluated by the number of survival days of mouse leukemia P388-bearing female CDF1 mice.

RESULTS: The chloroform extract of I. obliquus sclerotia inhibited proliferation of the P388 cells. Among the triterpenoids examined, only inotodiol inhibited P388 cell proliferation. DNA fragmentation and caspase-3/7 activation were observed in the P388 cells treated with inotodiol (30 microM). A caspase-3 inhibitor, DEVD-CHO (N-acetyl-Asp-Glu-Val-Asp-al, 100 microM) partially inhibited the DNA fragmentation and growth-inhibition induced by inotodiol. The intraperitoneal administration of 10 mg/kg inotodiol prolonged the number of survival days of the P388-bearing mice.

CONCLUSION: Inotodiol inhibits cell proliferation through apoptosis induction by activating caspase-3.

Antimutagenic effects of subfractions of Chaga mushroom (Inonotus obliquus) extract.

Mutat Res. 2009 Jan; 672(1):55-9. doi: 10.1016/j.mrgentox.2008.10.002. Epub 2008 Oct 17.

Inonotus obliquus is a mushroom commonly known as Chaga that is widely used in folk medicine in Siberia, North America, and North Europe. Here, we evaluated the antimutagenic and antioxidant capacities of subfractions of Inonotus obliquus extract. The ethyl acetate extract was separated by vacuum chromatography into three fractions, and the fraction bearing the highest antimutagenic activity was subsequently separated into four fractions by reversed phase (ODS-C18) column chromatography. The most antimutagenic fraction was then separated into two subfractions (subfractions 1 and 2) by normal phase silica gel column chromatography. Ames test analysis revealed that the subfractions were not mutagenic. At 50 μg/plate, subfractions 1 and 2 strongly inhibited the mutagenesis induced in Salmonella typhimurium strain TA100 by the directly acting mutagen MNNG (0.4 μg/plate) by 80.0% and 77.3%, respectively. They also inhibited 0.15 μg/plate 4NQO-induced mutagenesis in TA98 and TA100 by 52.6-62.0%. The mutagenesis in TA98 induced by the indirectly acting mutagens Trp-P-1 (0.15 μg/plate) and B(α)P (10 μg/plate) was reduced by 47.0-68.2% by the subfractions, while the mutagenesis in TA100 by Trp-P-1 and B(α)P was reduced by 70.5-87.2%. Subfraction 1 was more inhibitory than subfraction 2 with regard to the mutagenic effects of 4NQO, Trp-P-1, and B(α)P. Subfractions 1 and 2 also had a strong antioxidant activity against DPPH radicals and were identified by MS, 1H NMR and 13C NMR analyses as 3β-hydroxy-lanosta-8, 24-dien-21-al and inotodiol, respectively. Thus, we show that the 3beta-hydroxy-lanosta-8, 24-dien-21-al and inotodiol components of Inonotus obliquus bear antimutagenic and antioxidative activities.

Chaga mushroom (Inonotus obliquus) induces G0/G1 arrest and apoptosis in human hepatoma HepG2 cells.

World J Gastroenterol. 2008 Jan 28; 14(4):511-7.

AIM: To investigate the anti-proliferative and apoptotic effects of Chaga mushroom (Inonotus obliquus) water extract on human hepatoma cell lines, HepG2 and Hep3B cells.

METHODS: The cytotoxicity of Chaga extract was screened by 3-[4,5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide (MTT) assay. Morphological observation, flow cytometry analysis, Western blot were employed to elucidate the cytotoxic mechanism of Chaga extract.

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RESULTS: HepG2 cells were more sensitive to Chaga extract than Hep3B cells, as demonstrated by markedly reduced cell viability. Chaga extract inhibited the cell growth in a dose-dependent manner, which was accompanied with G0/G1-phase arrest and apoptotic cell death. In addition, G0/G1 arrest in the cell cycle was closely associated with down-regulation of p53, pRb, p27, cyclins D1, D2, E, cyclin-dependent kinase (Cdk) 2, Cdk4, and Cdk6 expression.

CONCLUSION: Chaga mushroom may provide a new therapeutic option, as a potential anticancer agent, in the treatment of hepatoma.

Lanostane-type triterpenoids from the sclerotia of Inonotus obliquus possessing anti-tumor promoting activity.

Eur J Med Chem. 2008 Nov; 43(11):2373-9. doi: 10.1016/j.ejmech.2008.01.037. Epub 2008 Feb 8.

Two new lanostane-type triterpenoids, 1 and 2 besides two known lanostane-type triterpenoids, 3 and 4 were isolated from the sclerotia of Inonotus obliquus. Their structures were determined to be lanosta-8,23E-diene-3beta,22R,25-triol (1) and lanosta-7:9(11),23E-triene-3beta,22R,25-triol (2) by spectral data. These compounds were tested for their anti-tumor-promoting activity using a short-term in vitro assay for EBV-EA activation induced by TPA. Compounds 1, 2 and 4 were stronger than the positive control, oleanolic acid. The most abundant compound 4 was investigated for the inhibitory effect in a two-stage carcinogenesis test on mouse skin using DMBA as an initiator and TPA as a promoter. Compound 4 was found to exhibit the potent anti-tumor promoting activity in the in vivo carcinogenesis test.

Identification of a novel blocker of IkappaBalpha kinase activation that enhances apoptosis and inhibits proliferation and invasion by suppressing nuclear factor-kappaB.

Mol Cancer Ther. 2008 Jan; 7(1):191-201. doi: 10.1158/1535-7163.MCT-07-0406.

3,4-dihydroxybenzalacetone (DBL) is a polyphenol derived from the medicinal plant Chaga [Inonotus obliquus (persoon) Pilat]. Although Chaga is used in Russia folk medicine to treat tumors, very little is known about its mechanism of action. Because most genes involved in inflammation, antiapoptosis, and cell proliferation are regulated by the transcription factor nuclear factor-kappaB (NF-kappaB), we postulated that DBL activity is mediated via modulation of the NF-kappaB activation pathway. We investigated the effects of DBL on NF-kappaB activation by electrophoretic mobility shift assay and on NF-kappaB-regulated gene expression by Western blot analysis. We found that DBL suppressed NF-kappaB activation by a wide variety of inflammatory agents, including tumor necrosis factor (TNF), interleukin-1beta, epidermal growth factor, okadaic acid, phorbol 12-myristate 13-acetate, and lipopolysaccharide. The suppression was not cell type specific and inhibited both inducible and constitutive NF-kappaB activation. DBL did not interfere with the binding of NF-kappaB to DNA but rather inhibited IkappaBalpha kinase activity, IkappaBalpha phosphorylation and degradation, p65 phosphorylation, and translocation. DBL also suppressed the expression of TNF-induced and NF-kappaB-regulated proliferative, antiapoptotic, and metastatic gene products. These effects correlated with enhancement of TNF-induced apoptosis and suppression of TNF-induced invasion. Together, our results indicate that DBL inhibits NF-kappaB activation and NF-kappaB-regulated gene expression, which may explain the ability of DBL to enhance apoptosis and inhibit invasion.

Structure determination of inonotsuoxides A and B and in vivo anti-tumor promoting activity of inotodiol from the sclerotia of Inonotus obliquus.

Bioorg Med Chem. 2007 Jan 1; 15(1):257-64. Epub 2006 Sep 30.

Two new lanostane-type triterpenoids, inonotsuoxides A (1) and B (2) along with three known lanostane-type triterpenoids, inotodiol (3), trametenolic acid (4), and lanosterol (5), were isolated from the sclerotia of Inonotus obliquus (Pers.: Fr.) (Japanese

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name: Kabanoanakake) (Russian name: Chaga). Their structures were determined to be 22R,25-epoxylanost-8-ene-3beta,24S-diol (1) and 22S,25-epoxylanost-8-ene-3beta,24S-diol (2) on the basis of spectral data including single crystal X-ray analysis. These compounds except for 2 were tested for their inhibitory effects on Epstein-Barr virus early antigen (EBV-EA) activation induced by 12-O-tetradecanoylphorbol-13-acetate (TPA), as a test for potential cancer chemopreventive agents. The most abundant triterpene, inotodiol (3), was investigated for the inhibitory effect in a two-stage carcinogenesis test on mouse skin using 7,12-dimethylbenz[a]anthracene (DMBA) as an initiator and TPA as a promoter. Compound 3 was found to exhibit the potent anti-tumor promoting activity in the in vivo carcinogenesis test.

Anti-cancer effect and structural characterization of endo-polysaccharide from cultivated mycelia of Inonotus obliquus.

Life Sci. 2006 May 30; 79(1):72-80. Epub 2006 Feb 3.

The endo-polysaccharide extracted from mycelia of Inonotus obliquus (Pers.:Fr.) Pil. (Hymenochaetaceae) is a specific activator of B cells and macrophages. However, the in vivo anti-cancer effects and the chemical structure of the endo-polysaccharide are unknown. We purified the endo-polysaccharide, investigated its anti-cancer effects via in vitro and in vivo assays, and performed a structural characterization. The endo-polysaccharide was extracted from I. obliquus mycelia cultivated in a 300-l pilot fermenter, followed by hot water extraction and ethanol precipitation. Purification was achieved by DEAE-cellulose ion-exchange chromatography and gel-permeation chromatography. Chemical analysis revealed that the purified endo-polysaccharide is an alpha-linked fucoglucomannan with a molecular weight of approximately 1,000 kDa. The anti-cancer activities of the endo-polysaccharide against various types of tumor cells were determined. No direct toxicity against either cancer or normal cells was observed. Intraperitoneal administration of the endo-polysaccharide significantly prolonged the survival rate of B16F10-implanted mice, resulting in a 4.07-fold increase in the survival rate at a dose of 30 mg/kg/day. After 60 days of feeding, approximately 67% of the initial number of mice survived with no tumor incidence based on macroscopic examination. These results indicate that the anti-cancer effect of endo-polysaccharide is not directly tumorcidal but rather is immuno-stimulating.

Reversal of the TPA-induced inhibition of gap junctional intercellular communication by Chaga mushroom (Inonotus obliquus) extracts: effects on MAP kinases.

Biofactors. 2006; 27(1-4):147-55.

Chaga mushroom (Inonotus obliquus) has continued to receive attention as a folk medicine with indications for the treatment of cancers and digestive diseases. The anticarcinogenic effect of Chaga mushroom extract was investigated using a model system of gap junctional intercellular communication (GJIC) in WB-F344 normal rat liver epithelial cells. The cells were pre-incubated with Chaga mushroom extracts (5, 10, 20 microg/ml) for 24 h and this was followed by co-treatment with Chaga mushroom extracts and TPA (12-O-tetradecanoylphorbol-13-acetate, 10 ng/ml) for 1 h. The inhibition of GJIC by TPA (12-O-tetradecanoylphorbol-13-acetate), promoter of cancer, was prevented with treatment of Chaga mushroom extracts. Similarly, the increased phosphorylated ERK1/2 and p38 protein kinases were markedly reduced in Chaga mushroom extracts-treated cells. There was no change in the JNK kinase protein level, suggesting that Chaga mushroom extracts could only block the activation of ERK1/2 and p38 MAP kinase. The Chaga mushroom extracts further prevented the inhibition of GJIC through the blocking of Cx43 phosphorylation. Indeed cell-to-cell communication through gap junctional channels is a critical factor in the life and death balance of cells because GJIC has an important function in maintaining tissue homeostasis through the regulation of cell growth, differentiation, apoptosis and adaptive functions of differentiated cells. Thus Chaga mushroom may act as a natural anticancer product by preventing the inhibition of GJIC through the inactivation of ERK1/2 and p38 MAP kinase.

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ANTI-MICROBIAL EFFECTS

Aqueous extract from a Chaga medicinal mushroom, Inonotus obliquus (higher Basidiomycetes), prevents herpes simplex virus entry through inhibition of viral-induced membrane fusion.

Int J Med Mushrooms. 2013; 15(1):29-38.

Chaga medicinal mushroom, Inonotus obliquus, a popular prescription in traditional medicine in Europe and Asia, was used to reduce inflammation in the nasopharynx and to facilitate breathing. The aqueous extract from I. obliquus (AEIO) exhibited marked decrease in herpes simplex virus (HSV) infection (the 50% inhibitory concentration was 3.82 µg/mL in the plaque reduction assay and 12.29 µg/mL in the HSV-1/blue assay) as well as safety in Vero cells (the 50% cellular cytotoxicity was > 1 mg/mL, and selection index was > 80). Using a time course assay, effective stage analysis, and fusion inhibition assay, the mechanism of anti-HSV activity was found against the early stage of viral infection through inhibition of viral-induced membrane fusion. Therefore, AEIO could effectively prevent HSV-1 entry by acting on viral glycoproteins, leading to the prevention of membrane fusion, which is different from nucleoside analog antiherpetics.

Antiviral activity of Inonotus obliquus fungus extract towards infection caused by hepatitis C virus in cell cultures.

Bull Exp Biol Med. 2011 Sep; 151(5):612-4.

Fractions of Inonotus obliquus fungus water extract exhibited a virucidal effect towards hepatitis C virus: it 100-fold reduced its infective properties within 10 min. The antiviral effects of fungus extracts manifested after preventive (24 h before infection) and therapeutic use (during infection of porcine embryo kidney cells). Moreover, the data indicate that the birch fungus extracts inhibit production of infective virus by porcine embryo kidney cells.

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ANTIOXIDATIVE AND ANTI-INFLAMMATORY EFFECTS

Antioxidant activity of Inonotus obliquus polysaccharide and its amelioration for chronic pancreatitis in mice.

Int J Biol Macromol. 2016 Jun; 87:348-56. doi: 10.1016/j.ijbiomac.2016.03.006. Epub 2016 Mar 5.

Inonotus obliquus polysaccharide (IOP) was extracted by water with a yield of 9.83% and purified by an anion-exchange DEAE cellulose column and Sephadex G-200 gel with a polysaccharide content of 98.6%. The scavenging activities for 2,2-diphenyl-1-picryl-hydrazyl (DPPH) and hydroxyl radicals of IOP were 82.3% and 81.3% respectively at a concentration of 5 mg/mL. IOP was composed of Man, Rha, Glu, Gal, Xyl and Ara in a molar ratio of 9.81:3.6 : 29.1 : 20.5 : 21.6 : 5.4 respectively. The gel permeation chromatography indicated that IOP was a homogeneous polysaccharide with molecular weight of 32.5 kDa. IOP helped to alleviate pancreatic acinar atrophy and weight loss for chronic pancreatitis (CP) mice induced by Diethyldithiocarbamate (DDC). The SOD level was increased most by IOP-H treatment (400 mg/kg body weight). MDA, IL-1β and LDH were significantly decreased by IOP treatment, especially hydroxyproline, IFN-γ and AMS levels were decreased 39.18%, 37.82% and 41.57% by IOP-H treatment respectively compared to MC group. In conclusion, IOP possessed strong antioxidant activity for scavenging free radicals in vitro and vivo which could be propitious to CP therapy in mice.

Investigation of three lignin complexes with antioxidant and immunological capacities from Inonotus obliquus.

Int J Biol Macromol. 2016 May; 86:587-93. doi: 10.1016/j.ijbiomac.2016.01.111. Epub 2016 Feb 1.

Mushroom Inonotus obliquus (I. obliquus), a folk medicine, has been widely used to treat several human malicious tumors since 16th century. In this study, three homogenous biomolecules (designated IOA1, IOA2 and IOA3) were prepared from the alkali extract of I. obliquus. Their molecular weights were measured to be 6.1 × 10(4), 2.9 × 10(4) and 3.5 × 10(4) g/mol respectively and all of them were characterized as lignin-carbohydrate complexes mainly comprised lignin as well as ~25% carbohydrates. Antioxidant assays indicated that all of them exhibited pronounced reductive power and strong scavenging activities on DPPH and hydroxyl radicals in vitro. Immunological tests showed that they could also significantly stimulate nitric oxide production and phagocytic activity in RAW 264.7 macrophages. These results implied that the lignin-carbohydrate complexes extracted from I. obliquus might be used as novel natural antioxidants or immunostimulants in functional foods or pharmaceutical candidates.

Phenolic compounds from the fungus Inonotus obliquus and their antioxidant properties.

J Antibiot (Tokyo). 2015 Jul 29. doi: 10.1038/ja.2015.83. [Epub ahead of print]

Polysaccharides from Inonotus obliquus sclerotia and cultured mycelia stimulate cytokine production of human peripheral blood mononuclear cells in vitro and their chemical characterization.

Int Immunopharmacol. 2014 Aug; 21(2):269-78. doi:10.1016/j.intimp. 2014.05.015. Epub 2014 May 24.

Inonotus obliquus is an edible and medicinal mushroom to treat many diseases. In the present study, polysaccharides and fractions were isolated and purified by DEAE-52 and Sephadex G-200 chromatography from I. obliquus wild sclerotia, culture broth and cultured mycelia under submerged fermentation. The extracts and fractions could significantly induce the secretion of TNF-α, IFN-γ, IL-1β, and IL-2 in human peripheral blood mononuclear cells (PBMCs) and showed no toxicity to PBMCs. The stimulation effect of the six extracts and eight fractions on the four-cytokine production was dose-dependent. Sclerotial polysaccharides were more effective in the four-cytokine production at 150 μg/ml while exopolysaccharides and

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endopolysacchrides showed a much better effect on IL-1β production at 30 μg/ml. Purified fractions from exopolysaccharides and endopolysaccharides were more effective than the fraction from sclerotia in most cytokine production. These heteropolysaccharide-protein conjugates mainly contained glucose, galactose, and mannose. Protein content, molecular weight, monosaccharide molar ratio, and anomeric carbon configuration differed from each other and had effects on the cytokine induction activity of the polysaccharides to some extent.

Anti-inflammatory and anticancer activities of extracts and compounds from the mushroom Inonotus obliquus.

Food Chem. 2013 Aug 15; 139(1-4):503-8. doi: 10.1016/j.foodchem. 2013.01.030. Epub 2013 Feb 1.

Mushroom Inonotus obliquus (I. obliquus) has been used as functional food and traditional Chinese herbs for long time. An efficient method for bioassay-guided preparative isolation was used for identifying the anti-inflammatory and anticancer constituents in I. obliquus. The petroleum ether and ethyl acetate fractions were found to have significant inhibition effects on NO production and NF-κB luciferase activity in macrophage RAW 264.7 cells and cytotoxicity against human prostatic carcinoma cell PC3 and breast carcinoma cell MDA-MB-231. Six main constituents were isolated from these two fractions and they were identified as lanosterol (1), 3β-hydroxy-8,24-dien-21-al (2), ergosterol (3), inotodiol (4), ergosterol peroxide (5) and trametenolic acid (6). Compound ergosterol, ergosterol peroxide and trametenolic acid showed anti-inflammatory activities and ergosterol peroxide and trametenolic acid showed obviously cytotoxicity on human prostatic carcinoma cell PC3 and breast carcinoma MDA-MB-231 cell. The results obtained in this work might contribute to understanding the biological activity of mushroom I. obliquus for food and drug application.

Chemical analysis and antioxidant activity of polysaccharides extracted from Inonotus obliquus sclerotia.

Int J Biol Macromol. 2013 Nov; 62:691-6. doi: 10.1016/j.ijbiomac.2013.10.016. Epub 2013 Oct 18.

Three water-soluble polysaccharide fractions (IOP40, IOP60 and IOP80) were isolated by using different concentrations of alcohol precipitation from Inonotus obliquus sclerotia. Their physicochemical properties, including total sugar content, protein content, monosaccharide composition and percentage were analyzed. And their in vitro antioxidant capacities were investigated in terms of reducing power assay and scavenging of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals, hydroxyl radicals, superoxide anion radicals and hydrogen peroxide (H2O2). In general, three polysaccharide fractions exhibited increasing antioxidant activity with increasing concentration at the ranges of tested dosage. The orders of reducing power, DPPH-scavenging capacity, H2O2-scavenging capacity, and hydroxyl-scavenging activity were all IOP60>IOP40>IOP80. These findings demonstrated that three polysaccharide fractions extracted from I. obliquus, especially IOP60, could be employed as natural ingredients in functional food and pharmaceutical industry to alleviate the oxidative stress.

Orally administered aqueous extract of Inonotus obliquus ameliorates acute inflammation in dextran sulfate sodium (DSS)-induced colitis in mice.

J Ethnopharmacol. 2012 Sep 28; 143(2):524-32. doi: 10.1016/j.jep.2012.07.008. Epub 2012 Jul 20.

ETHNOPHARMACOLOGICAL RELEVANCE: Chaga mushroom (Inonotus obliquus) has been used in folk medicine to treat several disorders through its various biological functions. I. obliquus is claimed to produce general immune-potentiating and strengthening, antiinflammatory, and antitumor properties, but its effects on intestinal inflammation (ulcerative colitis) are clearly not understood.

AIM OF THE STUDY: To determine the effects and mode of action of an aqueous extract of I. obliquus (IOAE) on experimental colitis in mice induced by dextran sulfate sodium (DSS).

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MATERIALS AND METHODS: Female 5-week-C57BL/6 mice were randomized into groups differing in treatment conditions (prevention and treatment) and doses of IOAE (50 and 100mg/kg body weight). Mice were exposed to DSS (2%) in their drinking water over 7 day to induce acute intestinal inflammation. In colon tissues, we evaluated histological changes by hematoxylin and eosin staining, levels of iNOS by immuno-histochemical staining, and neutrophil influx by myeloperoxidase assay. mRNA expression of pro-inflammatory mediators TNF-α, IL-1β, IL-6, and IFN-γ was determined by RT-PCR.

RESULTS: Histological examinations indicated that IOAE suppressed edema, mucosal damage, and the loss of crypts induced by DSS. IOAE markedly attenuated DSS-induced iNOS levels and myeloperoxidase accumulation in colon tissues, demonstrating its suppressive effect on infiltration of immune cells. In addition, IOAE significantly inhibited mRNA expression of pro-inflammatory cytokines induced by DSS in colon tissues.

CONCLUSION: Our results suggest anti-inflammatory effect of IOAE at colorectal sites due to down-regulation of the expression of inflammatory mediators. Suppression of TNF-α and iNOS together with IL-1β by IOAE denotes that it might be a useful supplement in the setting of inflammatory bowel disease.

Antioxidative properties of crude polysaccharides from Inonotus obliquus.

Int J Mol Sci. 2012; 13(7):9194-206. doi: 10.3390/ijms13079194. Epub 2012 Jul 23.

The mushroom Inonotus obliquus has been widely used as a folk medicine in Russia, Poland and most of the Baltic countries. In this study, water-soluble and alkali-soluble crude polysaccharides (IOW and IOA) were isolated from I. obliquus, and the carbohydrate-rich fractions IOW-1 and IOA-1 were obtained respectively after deproteination and depigmentation. Their contents, such as neutral carbohydrate, uronic acid and protein, were measured. Their antioxidant properties against chemicals-induced reactive species (ROS) including 1,1'-Diphenyl-2-picrylhydrazyl (DPPH) radical, hydroxyl radical and superoxide anion radical, as well as their protective effects on H(2)O(2)-induced PC12 cell death were investigated. Results showed that I. obliquus polysaccharides can scavenge all ROS tested above in a dose-dependent manner. IOA and its product IOA-1 could rescue PC12 cell viability from 38.6% to 79.8% and 83.0% at a concentration of 20μg/mL. Similarly, IOW and its product IOW-1 at the same dose, can also increase cell viability to 84.9% and 88.6% respectively. The antioxidative activities of water-soluble and alkali-soluble polysaccharide constituents from I. obliquus might contribute to diverse medicinal and nutritional values of this mushroom.

Antioxidant activities of five polysaccharides from Inonotus obliquus.

Int J Biol Macromol. 2012 Jun 1; 50(5):1183-7. doi: 10.1016/j.ijbiomac.2012.03.019. Epub 2012 Mar 30.

Five polysaccharides (IOP1b, IOP2a, IOP2c, IOP3a and IOP4) were isolated and purified from Inonotus obliquus by DEAE-Sepharose fast flow and SepharoseCL-6B column chromatography. Their chemical and physical characteristics were determined and antioxidant activities were investigated on the basis of hydroxyl radical assay, superoxide radical assay and ferric-reducing antioxidant power assay. The results showed that five polysaccharides exhibited antioxidant activities, and the higher content of uronic acid and proteinous substances, the stronger antioxidant activities of polysaccharides. Besides, molecular weights of polysaccharides also influence their antioxidant activities. IOP3a and IOP4 showed higher antioxidant properties than IOP1b, IOP2a and IOP2c.

Analysis of antioxidant metabolites by solvent extraction from sclerotia of Inonotus obliquus (Chaga).

Korean J Intern Med. 2013 Mar; 28(2):216-23. doi: 10.3904/kjim.2013.28.2.216. Epub 2013 Feb 27.

INTRODUCTION: The sclerotia of Inonotus obliquus (Chaga) are effective therapeutic agents to treat several human malignant tumours and other diseases without unacceptable toxic side-effects.

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OBJECTIVE: To investigate solvent effects on metabolic profiles and antioxidant activities of extracts of Chaga.

METHODOLOGY: Chaga was extracted by petroleum ether, chloroform, ethyl acetate, acetone, ethanol and water. Solvent effects on metabolites in the extracts were assayed by NMR-based metabolomic analysis. Antioxidant activities were indicated as capacities for scavenging superoxide anion, DPPH and hydroxyl radicals.

RESULTS: Petroleum ether and chloroform extracts contained primarily lanostane-type triterpenoids (LT), whereas the extracts of ethyl acetate, acetone and ethanol were characterised by the predominant presence of hispidin analogues and LT, and water extracts by polysaccharides and phenolic compounds. The ethyl acetate, acetone, ethanol and water extracts revealed remarkable potential for scavenging the tested radicals, while those of petroleum ether and chloroform did not. Polyphenols are the major contributors for quenching the tested free radicals, while in LT only compounds 16, 17 and 22 participated in scavenging hydroxyl radicals.

CONCLUSION: Polyphenols in Chaga are the principles for quenching free radicals while polysaccharides and a few LT compounds contribute partially in scavenging DPPH and hydroxyl radicals, respectively. NMR-based metabolomic analysis is a useful method by which to correlate ^{1}H-NMR spectra of Chaga extracts with their antioxidant activities, and this allows the prediction of potentials for scavenging free radicals by ^{1}H-NMR spectroscopy.

Anti-inflammatory effect of Inonotus obliquus, Polygala senega L., and Viburnum trilobum in a cell screening assay.

J Ethnopharmacol. 2009 Sep 25; 125(3):487-93. doi: 10.1016/j.jep.2009.06.026. Epub 2009 Jul 3.

AIM OF THE STUDY: The purpose of the study was to assess the anti-inflammatory effects of the mushroom Inonotus obliquus (Chaga), Polygala senega (Senega) and Viburnum trilobum (Cranberry) bark extract fractions from locally produced materials in lipopolysaccharide (LPS) induced murine macrophage RAW 164.7 cells.

MATERIALS AND METHODS: Four fractions from each of the three extracts were obtained: (80% ethanol extracted; Fa), (water-soluble polysaccharide fraction; Fb), (Polyphenolic fraction; Fc) and (ETOAc/H(2)O extracted fraction; Fd). These extract fractions were tested in the cell screening system at 50,100 and 500 microg/ml for their ability to inhibit LPS induced inflammatory cytokines IL-1beta, TNFalpha and IL-6. Supernatants from LPS alone treated cells were used as control. The cytokines in the cell culture supernatants following treatments with extract fractions were quantified by ELISA method, using 96 well ELISA plates.

RESULTS: All fractions of the extracts significantly inhibited ($p<0.05$) the levels of IL-1beta, IL-6 and TNFalpha except the polyphenolic Fc fraction of Senega which showed an increased production of IL-6. Furthermore, each fraction showed a dose-dependant anti-inflammatory effect. Nitric oxide production was not affected by cranberry and senega, while Chaga significantly reduced NO production in murine macrophage cell assay.

CONCLUSIONS: These results demonstrate that the extracts obtained from the root of Polygala senega L., bark of Viburnum trilobum, and the mushroom Inonotus obliquus possess anti-inflammatory properties when tested in a RAW 264.7 macrophage cell system.

Comparative study of antioxidant activity and antiproliferative effect of hot water and ethanol extracts from the mushroom Inonotus obliquus.

J Biosci Bioeng. 2009 Jan; 107(1):42-8. doi: 10.1016/j.jbiosc.2008.09.004.

The medicinal mushroom Inonotus obliquus is a traditional and widely used multi-functional fungus. Hot water (50 degrees C, 70 degrees C, and 80 degrees C) and ethanol crude extracts of I. obliquus were investigated for their antioxidant activity with

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superoxide dismutase (SOD) and (1,1-diphenyl-2-picryhydrazyl) (DPPH) radical-scavenging activity assays. We also investigated the antiproliferative effects and ability of the extracts to induce apoptosis in human colon cancer DLD-1 cells. Among the four extracts, the ethanol extract (EE) exhibited the strongest SOD-like activity and antiproliferative effect on DLD-1 cells, and exposure to the EE resulted in the induction of apoptosis, whereas no apoptosis was observed in DLD-1 cells exposed to the hot water extracts (HWEs). HWE at 70 degrees C (HWE70) exhibited the strongest DPPH radical-scavenging activity (EC50, 126 microg/ml), whereas the EE showed the weakest activity (EC50, 224 microg/ml). The different biological activities among the four extracts may be attributed to differences in their chemical composition, partially supported by polysaccharide, protein and phenolic content, and the 1H-NMR spectra.

Antioxidant activities of extracts and subfractions from Inonotus Obliquus.

Int J Food Sci Nutr. 2009; 60 Suppl 2:175-84. doi: 10.1080/09637480903042279. Epub 2009 Jul 1.

The ethanolic crude extracts and three subfractions (ethyl acetate fraction, n-butanol fraction, and aqueous fraction) from Inonotus Obliquus were obtained by sequential partitioning and their antioxidant activities were investigated in the present study. The methods of the total antioxidant capacity measured by the ferric-reducing antioxidant power assay, reducing power assay, scavenging activities towards DPPH, superoxide anion and hydroxyl radical employed in this study were established in in vitro systems. The amounts of total phenolics and total flavonoids were also determined by spectrophotometer. The results showed that the crude extracts and subfractions exhibited antioxidant activities in different evaluating system. The decreasing order of antioxidant activities is ethyl acetate fraction >n-butanol fraction >crude extract>aqueous fraction. A similar order of the amounts of phenolics and flavonoids in extract and subfractions was found. The results showed that the extent of antioxidant activities is in accordance with the amounts of phenolics and flavonoids existing in extracts and subfractions.

Prevention of hydrogen peroxide-induced oxidative stress in PC12 cells by 3,4- dihydroxybenzalacetone isolated from Chaga (Inonotus obliquus (persoon) Pilat).

Free Radic Biol Med. 2009 Oct 15; 47(8):1154-61. doi: 10.1016/j.freeradbiomed.2009.07.029. Epub 2009 Jul 30.

Chaga (Inonotus obliquus (persoon) Pilat) is a mushroom traditionally used as a folk medicine for tumors and stomach ulcers in Russia. Previously, we reported the antioxidant potential of Chaga extracts and seven isolated phenolic ingredients. In the present study, we investigated the protective effects of Chaga extracts and other isolated phenolic ingredients against H(2)O(2)-induced oxidative stress in PC12 cells. Intracellular generation of reactive oxygen species (ROS) leads to oxidative stress and subsequent damage of cellular and nuclear components. Chaga extracts and the phenolic ingredients, 3,4-dihydroxybenzalacetone (DBL) and caffeic acid (CA), effectively suppressed intracellular ROS level in H(2)O(2)-treated cells. The H(2)O(2)-induced cell death was more pronounced, effectively prevented in the cells treated with DBL than in cells treated with CA. In addition, ROS activate various signal transduction pathways including the mitogen-activated protein kinase (MAPK) cascade. Therefore, we examined the potentially beneficial effects of DBL on extracellular signal-regulated protein kinase (ERK), c-Jun NH(2)-terminal kinase (JNK), and p38-MAPK signaling activated by H(2)O(2) stimulation. DBL selectively inhibited the phosphorylation of p38-MAPK, without affecting JNK and ERK.

Accumulation of antioxidant phenolic constituents in submerged cultures of Inonotus obliquus.

Bioresour Technol. 2009 Feb; 100(3):1327-35. doi: 10.1016/j.biortech.2008.05.002. Epub 2008 Sep 27.

Phenolic compounds produced by sclerotia of Inonotus obliquus are the active constituents responsible for antioxidant activities. In this study, I. obliquus was grown in a continuously stirred tank reactor (CSTR) to explore how it accumulates phenolic compounds in different culture media and whether these compounds possess antioxidant activities. Phenolic

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compounds produced by I. obliquus in the control medium consisted of melanins, flavonoids, polyphenols and small phenolics. Their accumulation was affected by adding H(2)O(2) to the medium, where increased levels of total intracellular phenols (TIP) and melanins, but less total extracellular phenol (TEP) occurred. Simultaneous exposure to H(2)O(2) and arbutin resulted in a further increase in TIP production and reduced accumulation of TEP. Both TIP and TEP obtained at different culture ages and media were active in scavenging superoxide anion and DPPH radicals. Therefore, production of phenolic compounds by I. obliquus is enhanced by imposing oxidative stress, which might allow it to be exploited as a reliable source of pharmaceutically important phenolic compounds.

Identification of Inonotus obliquus and analysis of antioxidation and antitumor activities of polysaccharides.

Curr Microbiol. 2008 Nov; 57(5):454-62. doi: 10.1007/s00284-008-9233-6. Epub 2008 Sep 16.

Inonotus obliquus, a wild wood-decay fungus which grows on Betula trees in cool climates, has a variety of biological activities that the scientific community is paying more and more attention to. However, the research work is moving at a snail's pace. The methods of strain identification and the hypha microstructure have not been reported. We isolated one strain of filamentous molds from fruit body which was collected from birch wood on Changbai Mountain, cultivated mycelia on an inclined plane, and examined its micromorphology based on macroscopic examination. The strain was identified as I. obliquus by sequencing its ITS (internal transcribed spacer) domain. We subsequently investigated some of the mycelium polysaccharides' biological activities. The strain used in this study as the producers of antioxidation and anticancer polysaccharides was LNUF008. After fermentation in a 30-L fermenter, mycelia were obtained. The polysaccharides were extracted by transonic recirculation and ethanol precipitation. In order to identify the antioxidation effect, we designed an assay to test the inhibition of endogenous and Fe(2+)-Cys-induced lipid peroxidation as well as ferrous sulfate/ascorbate (Fe(2+)-VC)-induced mitochondrial swelling. The MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] method was used to study the antiproliferation activity of the polysaccharides on SMMC7721 hepatoma cells. The results indicate that I. obliquus polysaccharides exhibit high antitumor and antioxidation effects. The submerged culture method of growing I. obliquus will enable large-scale production of the polysaccharides.

Chaga mushroom extract inhibits oxidative DNA damage in lymphocytes of patients with inflammatory bowel disease.

Biofactors. 2007; 31(3-4):191-200.

Inflammatory Bowel Disease (IBD) is partly caused by oxidative stress from free radicals and reduced antioxidant levels. Using hydrogen peroxide to induce oxidative stress in vitro in peripheral lymphocytes we investigated the induction of DNA damage supplemented with ethanolic extract of Chaga mushroom as a protective antioxidant. Lymphocytes were obtained from 20 IBD patients and 20 healthy volunteers. For treatment, a constant H_{2}O_{2 } dose (50 microg/ml) was used with variable doses of Chaga extract (10-500 microg/ml). DNA damage was evaluated in 50 cells per individual and dose using the Comet assay (making 1000 observations per experimental point ensuring appropriate statistical power). Chaga supplementation resulted in a 54.9% (p < 0.001) reduction of H_{2}O_{2 } induced DNA damage within the patient group and 34.9% (p < 0.001) within the control group. Lymphocytes from Crohn's disease (CD) patients had a greater basic DNA damage than Ulcerative Colitis (UC) patients (p < 0.001). Conclusively, Chaga extract reduces oxidative stress in lymphocytes from IBD patients and also healthy individuals when challenged in vitro. Thus, Chaga extract could be a possible and valuable supplement to inhibit oxidative stress in general.

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New antioxidant polyphenols from the medicinal mushroom Inonotus obliquus.

Bioorg Med Chem Lett. 2007 Dec 15; 17(24):6678-81. Epub 2007 Oct 25.

The fruiting body of Inonotus obliquus, a medicinal mushroom called chaga, has been used as a traditional medicine for cancer treatment. Although this mushroom has been known to exhibit potent antioxidant activity, the mechanisms responsible for this activity remain unknown. In our investigation for free radical scavengers from the methanolic extract of this mushroom, inonoblins A (1), B (2), and C (3) were isolated along with the known compounds, phelligridins D (4), E (5), and G (6). Their structures were established by extensive spectroscopic analyses. These compounds exhibited significant scavenging activity against the ABTS radical cation and DPPH radical, and showed moderate activity against the superoxide radical anion.

Antioxidant small phenolic ingredients in Inonotus obliquus (persoon) Pilat (Chaga).

Chem Pharm Bull (Tokyo). 2007 Aug; 55(8):1222-6.

Inonotus obliquus (persoon) Pilat (Chaga, in Russia, kabanoanatake in Japan) is a fungus having been used as a folk medicine in Russia and said to have many health beneficial functions such as immune modulating and anti-cancer activities. In the present study, the antioxidant activity of hot water extract (decoction) of Chaga was precisely compared with those of other medicinal fungi (Agaricus blazei Mycelia, Ganoderma lucidum and Phellinus linteus) showing Chaga had the strongest antioxidant activity among fungi examined in terms of both superoxide and hydroxyl radicals scavenging activities. Further determination of the antioxidant potential of isolated fruiting body (brown part) and Sclerotium (black part) revealed the 80% MeOH extract of fruiting body had the highest potential as high as that of Chaga decoction. Finally, seven antioxidant components were isolated and purified from the 80% MeOH extract of Chaga fruiting body, and their chemical structures were determined as small phenolics as follows: 4-hydroxy-3,5-dimethoxy benzoic acid 2-hydroxy-1-hydroxymethyl ethyl ester (BAEE), protocatechic acid (PCA), caffeic acid (CA), 3,4-dihybenzaladehyde (DB), 2,5-dihydroxyterephtalic acid (DTA), syringic acid (SA) and 3,4-dihydroxybenzalacetone (DBL). Notably, BAEE was assigned as the new compound firstly identified from the natural source in the present study.

In vivo and in vitro anti-inflammatory and anti-nociceptive effects of the methanol extract of Inonotus obliquus.

J Ethnopharmacol. 2005 Oct 3; 101(1-3):120-8.

The mushroom Inonotus obliquus (Fr.) Pilát (Hymenochaetaceae), has been traditionally used for the treatment of gastrointestinal cancer, cardiovascular disease and diabetes in Russia, Poland and most of Baltic countries. This study was designed to investigate the anti-inflammatory and anti-nociceptive effects of the methanol extract from Inonotus obliquus (MEIO) in vivo and in vitro. MEIO (100 or 200 mg/(kgday), p.o.) reduced acute paw edema induced by carrageenin in rats, and showed analgesic activity, as determined by an acetic acid-induced abdominal constriction test and a hot plate test in mice. To reveal the mechanism of the anti-inflammatory effect of MEIO, we examined its effect on lipopolysaccharide (LPS)-induced responses in a murine macrophage cell line RAW 264.7. MEIO was found to significantly inhibit the productions of nitric oxide (NO), prostaglandin E2 (PGE2) and tumor necrosis factor-alpha (TNF-alpha) in LPS-stimulated RAW 264.7 macrophages. Consistent with these observations, MEIO potently inhibited the protein and mRNA expressions of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2). Furthermore, MEIO inhibited the LPS-induced DNA binding activity of nuclear factor-kappaB (NF-kappaB), and this was associated with the prevention of inhibitor kappaB degradation and a reduction in nuclear p65 protein levels. Taken together, our data indicate that the anti-inflammatory and anti-nociceptive properties of MEIO may be due to the inhibition of iNOS and COX-2 expression via the down-regulation of NF-kappaB binding activity.

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Chaga mushroom extract inhibits oxidative DNA damage in human lymphocytes as assessed by comet assay.

Biofactors. 2004; 21(1-4):109-12.

The Chaga mushroom (Inonotus obliquus) is claimed to have beneficial properties for human health, such as anti-bacterial, anti-allergic, anti-inflammatory and antioxidant activities. The antioxidant effects of the mushroom may be partly explained by protection of cell components against free radicals. We evaluated the effect of aqueous Chaga mushroom extracts for their potential for protecting against oxidative damage to DNA in human lymphocytes. Cells were pretreated with various concentrations (10, 50, 100 and 500 microg/mL) of the extract for 1 h at 37 degrees C. Cells were then treated with 100 microM of H2O2 for 5 min as an oxidative stress. Evaluation of oxidative damage was performed using single-cell gel electrophoresis for DNA fragmentation (Comet assay). Using image analysis, the degree of DNA damage was evaluated as the DNA tail moment. Cells pretreated with Chaga extract showed over 40% reduction in DNA fragmentation compared with the positive control (100 micromol H2O2 treatment). Thus, Chaga mushroom treatment affords cellular protection against endogenous DNA damage produced by H2O2.

Antioxidant effect of Inonotus obliquus.

J Ethnopharmacol. 2005 Jan 4; 96(1-2):79-85.

The mushroom Inonotus obliquus (Fr.) Pilát (Hymenochaetaceae), has been widely used as a folk medicine in Russia, Poland and most of the Baltic countries. The purpose of this study was to elucidate the antioxidant capacities of Inonotus obliquus. Four extracts from the fungus were evaluated for antioxidant activity against the 1,1-diphenyl-2-picrylhydrazyl (DPPH), superoxide, and peroxyl radicals. The polyphenolic extract had a strong antioxidant activity, and the extract containing triterpenoids and steroids presented a relatively strong antioxidant effect. The polysaccharide extract, however, was inactive. The protective effects of these four extracts were assessed against hydrogen peroxide-induced oxidative stress using a human keratinocyte cell line, HaCaT. Our results show that the polyphenolic extract protected these cells against hydrogen peroxide-induced oxidative stress, while the polysaccharide, triterpenoid and steroid extracts were ineffective. Additionally, the remnant polyphenolic and low molecular weight polysaccharide extracts showed a weakly protective effect at a concentration of 50 microg/ml. Our results indicate that Inonotus obliquus has the capacity to scavenge free radicals at concentrations higher than 5 microg/ml and that the polyphenolic extract can protect cells against oxidative stress.

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ANTI-VIRAL EFFECTS

Inhibition of Murine Norovirus and Feline Calicivirus by Edible Herbal Extracts.

Food Environ Virol. 2017 Mar;9(1):35-44. doi: 10.1007/s12560-016-9269-x. Epub 2016 Nov 2.

Human noroviruses (HuNoVs) cause foodborne and waterborne viral gastroenteritis worldwide. Because HuNoV culture systems have not been developed thus far, no available medicines or vaccines preventing infection with HuNoVs exist. Some herbal extracts were considered as phytomedicines because of their bioactive components. In this study, the inhibitory effects of 29 edible herbal extracts against the norovirus surrogates murine norovirus (MNV) and feline calicivirus (FCV) were examined. FCV was significantly inhibited to 86.89 ± 2.01 and 48.71 ± 7.38% by 100 μg/mL of Camellia sinensis and Ficus carica, respectively. Similarly, ribavirin at a concentration of 100 μM significantly reduced the titer of FCV by 77.69 ± 10.40%. Pleuropterus multiflorus (20 μg/mL) showed antiviral activity of 53.33 ± 5.77, and 50.00 ± 16.67% inhibition was observed after treatment with 20 μg/mL of Alnus japonica. MNV was inhibited with ribavirin by 59.22 ± 16.28% at a concentration of 100 μM. Interestingly, MNV was significantly inhibited with 150 μg/mL Inonotus obliquus and 50 μg/mL Crataegus pinnatifida by 91.67 ± 5.05 and 57.66 ± 3.36%, respectively. Treatment with 20 μg/mL Coriandrum sativum slightly reduced MNV by 45.24 ± 4.12%. The seven herbal extracts of C. sinensis, F. carica, P. multiflorus, A. japonica, I. obliquus, C. pinnatifida, and C. sativum may have the potential to control noroviruses without cytotoxicity.

Identification of Inonotus obliquus polysaccharide with broad-spectrum antiviral activity against multi-feline viruses.

Int J Biol Macromol. 2017 Feb; 95:160-167. doi: 10.1016/j.ijbiomac.2016.11.054. Epub 2016 Nov 16.

Inonotus obliquus polysaccharides (IOPs) are a potential drug for the prevention and treatment of cancer, cardiopathy, diabetes, AIDs, pancreatitis and other diseases. In this study, we found that IOP can act as a broad-spectrum antiviral drug against feline viruses in the in vitro experiment. Using cell models of feline calicivirus (FCV), we demonstrated that IOP treatment was capable of exhibiting anti-FCV strain F9 activity in cell-based assays and also showed low cytotoxicity. Investigation of the mechanism of action of the compound revealed that IOP treatment induces its inhibitory actions directly on virus particles through blocking viral binding/absorpting. The inhibitory activity against other FCV isolates from China was also identified. More importantly, we found that IOP exhibited broad-spectrum antiviral activity against the feline herpesvirus 1, feline influenza virus H3N2 and H5N6, feline panleukopenia virus and feline infectious peritonitis virus that can contribute to respiratory and gastrointestinal diseases in cats. These findings suggest that IOP may be a potential broad-spectrum antiviral drug against feline viruses.

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BRAIN HEALTH

3,4-dihydroxybenzalacetone protects against Parkinson's disease-related neurotoxin 6-OHDA through Akt/Nrf2/glutathione pathway.

J Cell Biochem. 2014 Jan; 115(1):151-60. doi: 10.1002/jcb.24643.

Oxidative stress is implicated in the pathogenesis of various neurodegenerative diseases including Parkinson's disease (PD). 3,4-Dihydroxybenzalacetone (DBL) is a small catechol-containing compound isolated from Chaga (Inonotus obliquus [persoon] Pilat), and has been reported to have beneficial bioactivities, including antioxidative, anti-inflammatory, and anti-tumorigenic activities, with a relatively low toxicity to normal cells. We, therefore, investigated the neuroprotective activity of DBL against the PD-related neurotoxin 6-hydroxydopamine (6-OHDA). Pretreatment of human neuroblastoma SH-SY5Y cells with DBL, but not with another Chaga-derived catechol-containing compound, caffeic acid, dose-dependently improved the survival of 6-OHDA-treated cells. Although DBL did not reduce 6-OHDA-induced reactive oxygen species in the cell-free system, it promoted the translocation of Nrf2 to the nucleus, activated the transcription of Nrf2-dependent antioxidative genes, and increased glutathione synthesis in the cells. Buthionine sulfoximine, an inhibitor of glutathione synthesis, but not Sn-mesoporphyrin IX, a heme oxygenase-1 inhibitor, or dicoumarol, an NAD(P)H:quinone oxidoreductase 1 inhibitor, abolished the protective effect of DBL against 6-OHDA. Furthermore, DBL activated stress-associated kinases such as Akt, ERK, and p38 MAPK, and PI3K or Akt inhibitors, but not ERK, p38, or JNK inhibitors, diminished DBL-induced glutathione synthesis and protection against 6-OHDA. These results suggest that DBL activates the Nrf2/glutathione pathway through PI3K/Akt, and improves survival of SH-SY5Y cells against 6-OHDA toxicity.

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IMMUNOMODULATION

Structural characterization of bioactive heteropolysaccharides from the medicinal fungus Inonotus obliquus (Chaga).

Carbohydr Polym. 2018 Apr 1; 185:27-40. doi: 10.1016/j.carbpol.2017.12.041. Epub 2017 Dec 20.

The aim of this paper was to perform a comprehensive characterization of polysaccharides isolated from the interior (IOI) and exterior (IOE) parts of the fungus Inonotus obliquus. Pre-extraction with DCM and MeOH, followed by water and alkali extraction and ethanol precipitation gave two water extracts and two alkali extracts. Neutral and acidic polysaccharide fractions were obtained after anion-exchange chromatography of the water extracts. The neutral polysaccharides (60-73 kDa) were heterogeneous and branched and consisted of a (1 → 3)-linked β-Glc backbone with (1 → 6)-linked kinks in the chain at approximately every fifth residue, with branches of (1 → 6)-linked β-Glc in addition to substantial amounts of (1 → 6)-linked α-Gal with 3-O-methylation at about every third Gal residue. The acidic polysaccharide fractions (10-31 kDa) showed similar structural motifs as the neutral fractions differing mainly by the presence of (1 → 4)-linked α-GalA and α-GlcA. β-Xyl, α-Man and α-Rha were also present in varying amounts in all fractions. No major structural differences between the IOI and IOE fractions were observed. An alkaline polysaccharide fraction (>450 kDa) was obtained from the IOI alkali extract and consisted mainly of (1 → 3)- and (1 → 6)-linked β-Glc and (1 → 4)-linked β-Xyl. Several of the fractions showed in vitro immunomodulatory effect by increasing NO production in the murine macrophage and dendritic cell lines J774.A1 and D2SC/1. Most fractions managed to increase NO production only at the highest concentration tested (100 µg/ml), while the neutral fraction IOE-WN activated potent NO production at 10 µg/ml and was considered the most promising immunomodulating fraction in this study.

Growth-Inhibitory and Immunomodulatory Activities of Wild Mushrooms from North-Central British Columbia (Canada).

Int J Med Mushrooms. 2017;19(6):485-497. doi: 10.1615/IntJMedMushrooms.v19.i6.10.

Wild mushrooms, especially from North America, have not been systematically explored for their medicinal properties. Here we report screening for the growth-inhibitory and immunomodulatory activities of 12 species collected from multiple locations in north-central British Columbia, Canada. Mushrooms were characterized using morphology and DNA sequencing, followed by chemical extraction into 4 fractions using 80% ethanol, 50% methanol, water, and 5% sodium hydroxide. Growth-inhibitory, immunostimulatory, and anti-inflammatory activities of 5 mushrooms (Leucocybe connata, Trichaptum abietinum, Hydnellum sp., Gyromitra esculenta, and Hericium coralloides) are reported here, to our knowledge for the first time. Growth-inhibitory effects were assessed using the cytotoxic MTT assay. Immunostimulatory activity was assessed by tumor necrosis factor-α production in Raw 264.7 macrophages, whereas anti-inflammatory activity was assessed based on the inhibition of lipopolysaccharide-induced tumor necrosis factor-α production. The ethanol and aqueous extracts of Hydnellum sp. were potent growth inhibitors, with a half-maximal inhibitory concentration of 0.6 mg/mL. All 5 fungi displayed strong immunostimulatory activity, whereas only L. connata and T. abietinum showed strong anti-inflammatory activity. For the 7 other fungi investigated, which included well-known medicinal species such as Inonotus obliquus, Phellinus igniarius, and Ganoderma applanatum, the remarkable similarities in the biological activities reported here, and by others for specimens collected elsewhere, suggest that mushrooms can produce similar metabolites regardless of their habitat or ecosystem. This is to our knowledge the first study to explore wild mushrooms from British Columbia for biological activities that are relevant to cancer, and the results provide an initial framework for the selection of mushroom species with the potential for discovery of novel anticancer compounds.

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The mast cell stabilizing activity of Chaga mushroom critical for its therapeutic effect on food allergy is derived from inotodiol.

Int Immunopharmacol. 2018 Jan; 54:286-295. doi: 10.1016/j.intimp.2017.11.025. Epub 2017 Nov 24.

While an anti-allergic effect of Chaga mushroom (Inonotus obliquus) has been indicated, its therapeutic effect on allergy and immunoregulatory mechanisms and chemical constituents directly responsible for that are hardly known. We examined the effect of 70% ethanol extract of Chaga mushroom (EE) and its dichloromethane (DF) and aqueous (AF) fractions using a mouse model of chicken ovalbumin (cOVA)-induced food allergy, and found that only EE and DF ameliorated allergy symptoms to a significant extent. The in vivo mast cell-stabilizing activity was also found only in EE and DF whereas the activities to suppress Th2 and Th17 immune responses and cOVA-specific IgE production in the small intestine were observed in all three treatment regimens, implying that inhibition of the mast cell function by lipophilic compounds was vital for the therapeutic effect. Results also indicated that inotodiol, a triterpenoid predominantly present in DF, played an active role as a mast cell stabilizer.

Chemical characterization and biological activity of Chaga (Inonotus obliquus), a medicinal "mushroom".

J Ethnopharmacol. 2015 Mar 13; 162:323-32. doi: 10.1016/j.jep.2014.12.069. Epub 2015 Jan 7.

ETHNOPHARMACOLOGICAL RELEVANCE: In Russian traditional medicine, an extract from the mushroom Inonotus obliquus (Fr.) Pil´at is used as an anti-tumor medicine and diuretic. It has been reported that Inonotus obliquus has therapeutic effects, such as anti-inflammatory, immuno-modulatory and hepatoprotective effects. This study was designed to investigate the chemical composition and biological properties of aqueous and ethanolic extracts of Inonotus obliquus from Finland, Russia, and Thailand. Their antioxidative, antimicrobial, and antiquorum properties were tested as well as the cytotoxicity on various tumor cell lines.

MATERIALS AND METHODS: The tested extract was subjected to conventional chemical study to identified organic acids and phenolic compounds. Antioxidative activity was measured by several different assays. Antimicrobial potential of extracts was tested by microdilution method, and antiquorum sensing activity and antibiofilm formation of Inonotus obliquus extracts was tested on Pseudomonas aeruginosa. Cytotoxicity of the extracts was tested on tumor cells (MCF-7, NCI-H460, HeLa and HepG2) and non-tumor liver cells primary cultures.

RESULTS: Oxalic acid was found as the main organic acid, with the highest amount in the aqueous extract from Russia. Gallic, protocatechuic and p-hydroxybenzoic acids were detected in all samples. Inonotus obliquus extracts showed high antioxidant and antimicrobial activity. Extracts were tested at subMIC for anti-quorum sensing (AQS) activity in Pseudomonas aeruginosa and all extracts showed definite AQS activity. The assays were done using twitching and swarming of bacterial cultures, and the amount of produced pyocyanin as QS parameters. All the extracts demonstrated cytotoxic effect on four tumor cell lines and not on primary porcine liver cells PLP2.

CONCLUSIONS: As the Inonotus obliquus presence in Chaga conks is limited, further purification is necessary to draw quantitative conclusions. The presence of AQS activity in medicinal mushrooms suggests a broader anti-infectious disease protection than only immunomodulatory effects.

Antitumor and immunomodulatory activity of water-soluble polysaccharide from Inonotus obliquus.

Carbohydr Polym. 2012 Oct 1; 90(2):870-4. doi: 10.1016/j.carbpol.2012.06.013. Epub 2012 Jun 17.

The medicinal mushroom Inonotus obliquus has been used as a folk remedy for a long time in Russia and East-European countries to treat gastrointestinal cancer, cardiovascular disease and diabetes. In our study, a water-soluble polysaccharide (ISP2a) was successfully purified from I. obliquus by DEAE-Sepharose CL-6B and Sepharose CL-6B column chromatography. In vivo ISP2a had not only shown antitumor activity, but also could significantly enhance the immune response of tumor-bearing

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mice. In addition, ISP2a significantly enhanced the lymphocyte proliferation and increased the production of TNF-α. Results of these studies demonstrated that ISP2a had a potential application as natural antitumor agent with immunomodulatory activity.

Inonotus obliquus containing diet enhances the innate immune mechanism and disease resistance in olive flounder Paralichythys olivaceus against Uronema marinum.

Fish Shellfish Immunol. 2012 Jun; 32(6):1148-54. doi: 10.1016/j.fsi.2012.03.021. Epub 2012 Mar 30.

The present study describes the effect of diet supplementation with Chaga mushroom, Inonotus obliquus extract at 0%, 0.01%, 0.1%, and 1.0% levels on the innate humoral (lysozyme, antiprotease, and complement), cellular responses (production of reactive oxygen and nitrogen species and myeloperoxidase), and disease resistance in olive flounder, Paralichythys olivaceus against Uronema marinum. The lysozyme activity and complement activity significantly increased in each diet on weeks 2 and 4 against pathogen. The serum antiprotease activity and reactive nitrogen intermediates production significantly increased in fish fed with 0.1% and 1.0% diets from weeks 1-4. However, reactive oxygen species production and myeloperoxidase activity significantly increased in 1.0% and 2.0% diets on weeks 2 and 4. In fish fed with 0.1% and 1.0% diets and challenged with U. marinum the cumulative mortality was 50% and 40% while in 0% and 0.01% diets the mortality was 85% and 55%. The results clearly indicate that supplementation diet with I. obliquus at 0.1% and 1.0% level positively enhance the immune system and confer disease resistance which may be potentially used as an immunoprophylactic in finfish culture.

Inonotus obliquus extracts suppress antigen-specific IgE production through the modulation of Th1/Th2 cytokines in ovalbumin-sensitized mice.

J Ethnopharmacol. 2011 Oct 11; 137(3):1077-82. doi: 10.1016/j.jep.2011.07.024. Epub 2011 Jul 28.

ETHNOPHARMACOLOGICAL RELEVANCE: Chaga mushroom (Inonotus obliquus, IO) has been used as a folk remedy for cancer, digestive system diseases, and other illnesses in Russia and Eastern Europe.

AIM OF THE STUDY: In the present study, we investigated the immunomodulating effects of IO through in vivo and ex vivo studies.

MATERIALS AND METHODS: Serum immunoglobulins (IgE, IgG(1), and IgG(2a)) and cytokines (interleukin (IL)-4, interferon (IFN)-γ, and IL-2) were measured in concanavalin A (ConA)-stimulated splenocytes and CD4(+) T cells. The nitric oxide (NO) secretion of lipopolysaccharide (LPS)-stimulated peritoneal macrophages was also measured after oral administration of 50, 100, or 200 mg kg(-1) d(-1) IO hot water extract (IOE) to ovalbumin (OVA)-sensitized BALB/c mice.

RESULTS: We found that the OVA-induced increase in serum IgE and IgG(2a) was significantly suppressed when IOE was orally administered after the second immunization with OVA. ConA stimulation in spleen cells isolated from OVA-sensitized mice treated with 100 mg kg(-1) IOE resulted in a 25.2% decrease in IL-4 production and a 102.4% increase in IFN-γ, compared to the controls. Moreover, IL-4, IFN-γ, and IL-2 were significantly reduced after ConA stimulation in isolated CD4(+)T cells. We also determined that IOE inhibits the secretion of NO from LPS-stimulated peritoneal macrophages ex vivo.

CONCLUSIONS: We suggest that IO modulates immune responses through secretion of Th1/Th2 cytokines in immune cells and regulates antigen-specific antibody production.

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Immunostimulating activity by polysaccharides isolated from fruiting body of Inonotus obliquus.

Mol Cells. 2011 Feb; 31(2):165-73. doi: 10.1007/s10059-011-0022-x. Epub 2010 Dec 22.

In this study, we investigated the immunostimulating activity of polysaccharides isolated from fruiting body of Inonotus obliquus (PFIO). Additionally, the signaling pathway of PFIO-mediated macrophage activation was investigated in RAW264.7 macrophage cells. We found that PFIO was capable of promoting NO/ROS production, TNF-α secretion and phagocytic uptake in macrophages, as well as cell proliferation, comitogenic effect and IFN-γ/IL-4 secretion in mouse splenocytes. PFIO was able to induce the phosphorylation of three MAPKs as well as the nuclear translocation of NF-κB, resulting in activation of RAW264.7 macrophages. PFIO also induced the inhibition of TNF-α secretion by anti-TLR2 mAb, consequently, PFIO might be involved in TNF-α secretion via the TLR2 receptor. In addition, our results showed that oral administration of PFIO suppressed in vivo growth of melanoma tumor in tumorbearing mice. In conclusion, our experiments presented that PFIO effectively promotes macrophage activation through the MAPK and NF-κB signaling pathways, suggesting that PFIO may potentially regulate the immune response.

Anti-inflammatory effects of Inonotus obliquus in colitis induced by dextran sodium sulfate.

J Biomed Biotechnol. 2010; 2010:943516. doi: 10.1155/2010/943516. Epub 2010 Mar 10.

A total of 28 male BALB/c mice (average weight 20.7 +/- 1.6 g) were divided into 4 treatment groups and fed a commercial diet (A), a commercial diet + induced colitis by dextran sodium sulfate (DSS) (B), Inonotus obliquus (IO) administration (C), and IO administration + induced colitis by DSS (D). IO treatment (C, D) decreased the expression of tumor necrosis factor (TNF)-alpha and signal transducers and activators of transcription (STAT)1 compared to those of the colitis induced group (B). The expressions of IL-4 and STAT6 were decreased in group D compared to the colitis induced group (B). The serum immunoglobulin (Ig)E level decreased in IO treatment groups (C, D) compared to no IO treatment groups (A and B) although there was no significant difference between the IO treatment groups. Extract from IO itself had a weak cytotoxic effect on murine macrophage cell line (RAW264.7 cells). Extract from IO inhibited lipopolysaccharide- (LPS-) induced, TNF-alpha, STAT1, pSTAT1, STAT6, and pSTAT6 production in RAW264.7 cells.

Ethanol extract of Inonotus obliquus inhibits lipopolysaccharide-induced inflammation in RAW 264.7 macrophage cells.

J Med Food. 2007 Mar; 10(1):80-9.

Inonotus obliquus (Pers.:Fr.) Pil. is a white rot fungus that belongs to the family Hymenochaetaceae of Basidiomycetes. Extracts and fractions of this fungus have been known to have biological activities, including antimutagenic, anticancer, antioxidative, and immunostimulating effects. Recently, there have been reports that the anti-inflammatory and antinociceptive properties of the methanol extract of I. obliquus may be due to the inhibition of inducible nitric oxide (NO) synthase (iNOS) and cyclooxygenase-2 (COX-2) expression via the down-regulation of nuclear factor kappaB (NF-kappaB) binding activity. However, the effects of I. obliquus on Akt and mitogen-activated protein kinase (MAPK) activation of inflammatory mediator production have not yet been elucidated. In the present study, a 70% ethanol extract of I. obliquus (IOE70) showed antioxidative effects. We also tested the ability of the I. obliquus extract to inhibit the inflammatory cascades in lipopolysaccharide (LPS)-induced RAW 264.7 macrophage cells. The NO inhibition of IOE70 was better than that of other ethanol extracts from I. obliquus. To investigate the mechanism by which IOE 70 inhibits NO production and iNOS and COX-2 expression, we examined the activations of IkappaBalpha, Akt, and c-Jun NH(2) -terminal kinase (JNK) in LPS-activated macrophages. IOE70 markedly inhibited the phosphorylation of IkappaBalpha, Akt, and MAPKs in dose-dependent manners in LPS-activated macrophages. Taken together, these experiments demonstrated that IOE70 inhibition of LPS-induced expression of iNOS and COX-2 protein is mediated by Akt and JNK. Based on our findings, the most likely mechanism that can account for this biological effect of IOE70

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involves the inhibition of NF-kappaB through the phosphatidylinositol 3-kinase/Akt/IkappaB pathway and the inhibition of JNK activation. Thus, IOE70 might have useful clinical applications in the management of inflammatory diseases and may also be useful as a medicinal food.

Immunomodulatory Activity of the Water Extract from Medicinal Mushroom Inonotus obliquus.

Mycobiology. 2005 Sep; 33(3):158-62. doi: 10.4489/MYCO.2005.33.3.158. Epub 2005 Sep 30.

The immunomodulatory effect of aqueous extract of Inonotus obliquus, called as Chaga, was tested on bone marrow cells from chemically immunosuppressed mice. The Chaga water extract was daily administered for 24 days to mice that had been treated with cyclophosphamide (400 mg/kg body weight), immunosuppressive alkylating agent. The number of colony-forming unit (CFU)-granulocytes/macrophages (GM) and erythroid burst-forming unit (BFU-E), increased almost to the levels seen in non-treated control as early as 8 days after treatment. Oral administration of the extract highly increased serum levels of IL-6. Also, the level of TNF-α was elevated by the chemical treatment in control mice, whereas was maintained at the background level in the extract-treated mice, indicating that the extract might effectively suppress TNF-α related pathologic conditions. These results strongly suggest the great potential of the aqueous extract from Inonotus obliquus as immune enhancer during chemotherapy.

Immuno-stimulating effect of the endo-polysaccharide produced by submerged culture of Inonotus obliquus.

Life Sci. 2005 Sep 23; 77(19):2438-56.

Inonotus obliquus BELYU1102 was selected from 12 different strains of Inonotus as a producer of immuno-stimulating polysaccharide. After a batch fermentation of I. obliquus BELYU1102 was carried out in a 300 l pilot vessel, endo-polysaccharide and exo-polysaccharide were both obtained. The proliferation activity of endo-polysaccharide for splenic cells was much higher than the activity of exo-polysaccharide. The active endo-polysaccharide was produced primarily during the late stationary phase. Enhanced proliferation and polyclonal IgM antibody production were observed in B cells by purified water-soluble endo-polysaccharide. Nitrite production and expression of IL-1beta, IL-6, TNF-alpha, and iNOS in macrophages were also enhanced. However, the endo-polysaccharide did not affect the proliferation of T cells, the IL-2 expression of Th1 cells, or the IL-4 expression of Th2 cells. The endo-polysaccharide showed activities similar to lipopolysaccharide (LPS) for B cells and macrophages, but there was a large difference between the two polysaccharides because cellular activations induced by endo-polysaccharide were not affected by polymyxin B, a specific inhibitor of LPS. The endo-polysaccharide appeared to have other cellular binding sites with TLR-4 and did not show a direct toxicity against tumor cells. However, indirect anti-cancer effects via immuno-stimulation were observed. The mycelial endo-polysaccharide of I. obliquus is a candidate for use as an immune response modifier. Submerged mycelial cultures are advantageous for industrial production of polysaccharides.

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LIVER, RENAL AND PANCREATIC HEALTH

Actions of Inonotus obliquus against Hyperuricemia through XOD and Bioactives Screened by Molecular Modeling.

Int J Mol Sci. 2018 Oct 18;19(10). pii: E3222. doi: 10.3390/ijms19103222.

Inonotus obliquus is an edible mushroom and also a remedy against various diseases, especially metabolic syndrome. In this paper we report the actions of an ethanol extract of I. obliquus (IOE) against hyperuricemia in hyperuricemic mice, and the screen of bioactives. The extract (IOE) was prepared by extracting I. obliquus at 65 °C with ethanol, and characterized by HPLC. IOE at low, middle, and high doses reduced serum uric acid (SUA) of hyperuricemic mice (353 μmol/L) to 215, 174, and 152 μmol/L ($p < 0.01$), respectively, showing similar hypouricemic effectiveness to the positive controls. IOE showed a non-toxic impact on kidney and liver functions. Of note, IOE suppressed xanthine oxidase (XOD) activity in serum and liver, and also down-regulated renal uric acid transporter 1 (URAT1). Four compounds hit highly against XOD in molecular docking. Overall, the four compounds all occupied the active tunnel, which may inhibit the substrate from entering. The IC50 of betulin was assayed at 121.10 ± 4.57 μM, which was near to that of allopurinol (148.10 ± 5.27 μM). Betulin may be one of the anti-hyperuricemia bioactives in I. obliquus.

The polysaccharide from Inonotus obliquus protects mice from Toxoplasma gondii-induced liver injury.

Int J Biol Macromol. 2018 Nov 13. pii: S0141-8130(18)33881-9. doi: 10.1016/j.ijbiomac.2018.11.114. [Epub ahead of print]

The study aimed to explore the protective effects and mechanism of Inonotus obliquus polysaccharide (IOP) on liver injury caused by Toxoplasma gondii (T. gondii) infection in mice. The results showed that treatment with IOP significantly decreased the liver coefficient, the levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), malondialdehyde (MDA) and nitric oxide (NO), and increased the contents of antioxidant enzyme superoxide dismutase (SOD) and glutathione (GSH). IOP effectively decreased the expression of serum tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6), interleukin-1β (IL-1β), interferon-γ (IFN-γ) and interluekin-4 (IL-4) in T. gondii-infected mice. In agreement with these observations, IOP also alleviated hepatic pathological damages caused by T. gondii. Furthermore, we found that IOP down-regulated the levels of toll-like receptor 2 (TLR2) and toll-like receptor 4 (TLR4), phosphorylations of nuclear factor-κappaB (NF-κB) p65 and inhibitor kappaBα (IκBα), whereas up-regulated the expressions of nuclear factor erythroid 2-related factor 2 (Nrf2) and heme oxygenase-1 (HO-1). These findings suggest that IOP possesses hepatoprotective effects against T. gondii-induced liver injury in mice, and such protection is at least in part due to its anti-inflammatory effects through inhibiting the TLRs/NF-κB signaling axis and the activation of an antioxidant response by inducing the Nrf2/HO-1 signaling.

Inonotus obliquus polysaccharide regulates gut microbiota of chronic pancreatitis in mice.

AMB Express. 2017 Dec;7(1):39. doi: 10.1186/s13568-017-0341-1. Epub 2017 Feb 14.

Polysaccharide is efficient in attenuation of metabolic ailments and modulation of gut microbiota as prebiotics. The therapeutic effect of Inonotus obliquus polysaccharide (IOP) on chronic pancreatitis (CP) in mice has been validated in our previous study. However, it is not clear whether IOP is conducive to maintaining the homeostasis between gut microbiota and host. The aim of this study is to testify the potential effects of IOP on gut microbiota composition and diversity in mice with CP. The changes in glutathione peroxidase (GSH-PX), total antioxidant capacity (TAOC), tumor necrosis factor alpha (TNF-α), transforming growth factor beta (TGF-β), lipase and trypsin levels were measured by commercial assay kits, meanwhile the gut microbiota composition and diversity were analyzed by high throughput sequencing. The IOP treatment increased GSH-PX and TAOC levels,

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and decreased TNF-α, TGF-β, lipase and trypsin levels in CP mice. It was also observed that gut microbiota in IOP treated groups were less diverse than others in terms of lower Shannon diversity index and Chao 1 estimator. IOP increased the proportion of Bacteroidetes and decreased that of Firmicutes at phylum level. Bacteroidetes was found positively correlated with GSH-PX and TAOC, and Firmicutes correlated with TNF-α, TGF-β, and lipase. In conclusion, administration of IOP could regulate gut microbiota composition and diversity to a healthy profile in mice with CP, and some bacterial phylum significantly correlated with characteristic parameters.

Effects of polysaccharides isolated from Inonotus obliquus against hydrogen peroxide-induced oxidative damage in RINm5F pancreatic β-cells.

Mol Med Rep. 2016 Nov;14(5):4263-4270. doi: 10.3892/mmr.2016.5763. Epub 2016 Sep 22.

The purpose of the present study was to elucidate the cytoprotective effects of polysaccharides isolated from Inonotus obliquus. The polysaccharides were extracted from the fruiting body of I. obliquus (PFIO) and the liquid culture broth of I. obliquus (PLIO). The effects of PFIO and PLIO on hydrogen peroxide (H2O2) -induced oxidative damage of RINm5F pancreatic β-cells were comparatively investigated using an MTT assay, immunofluorescent staining, flow cytometry, and western blot analyses in vitro. The results of the present study demonstrated that treatment with PFIO and PLIO decreased DNA fragmentation and the rate of apoptosis. In addition, pretreatment of cells with PFIO and PLIO prior to H2O2 exposure resulted in increased insulin secretion and scavenging activity for intracellular reactive oxygen species, as compared with treatment with H2O2 alone. The results of the present study suggested that PFIO and PLIO may exert protective effects against H2O2-induced oxidative stress via the regulation of mitogen-activated protein kinases, nuclear factor-κB and apoptotic proteins. Therefore, PFIO and PLIO may have potential merit as a medicinal food for the prevention of diabetes.

Renal Protective Effects of Low Molecular Weight of Inonotus obliquus Polysaccharide (LIOP) on HFD/STZ-Induced Nephropathy in Mice.

Int J Mol Sci. 2016 Sep 13;17(9). pii: E1535. doi: 10.3390/ijms17091535.

Diabetic nephropathy (DN) is the leading cause of end-stage renal disease in diabetes mellitus. Oxidative stress, insulin resistance and pro-inflammatory cytokines have been shown to play an important role in pathogeneses of renal damage on type 2 diabetes mellitus (DM). Inonotus obliquus (IO) is a white rot fungus that belongs to the family Hymenochaetaceae; it has been used as an edible mushroom and exhibits many biological activities including anti-tumor, anti-oxidant, anti-inflammatory and anti-hyperglycemic properties. Especially the water-soluble Inonotus obliquus polysaccharides (IOPs) have been previously reported to significantly inhibit LPS-induced inflammatory cytokines in mice and protect from streptozotocin (STZ)-induced diabetic rats. In order to identify the nephroprotective effects of low molecular weight of IOP fraction (LIOP), from the fruiting bodies of Inonotus obliquus, high-fat diet (HFD) plus STZ-induced type 2-like diabetic nephropathy C57BL/6 mice were investigated in this study. Our data showed that eight weeks of administration of 10-100 kDa, LIOP (300 mg/kg) had progressively increased their sensitivity to glucose (less insulin tolerance), reduced triglyceride levels, elevated the HDL/LDL ratio and decreased urinary albumin/creatinine ratio(ACR) compared to the control group. By pathological and immunohistochemical examinations, it was indicated that LIOP can restore the integrity of the glomerular capsules and increase the numbers of glomerular mesangial cells, associated with decreased expression of TGF-β on renal cortex in mice. Consistently, three days of LIOP (100 μg/mL) incubation also provided protection against STZ + AGEs-induced glucotoxicity in renal tubular cells (LLC-PK1), while the levels of NF-κB and TGF-β expression significantly decreased in a dose-dependent manner. Our findings demonstrate that LIOP treatment could ameliorate glucolipotoxicity-induced renal fibrosis, possibly partly via the inhibition of NF-κB/TGF-β1 signaling pathway in diabetic nephropathy mice.

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Hepatoprotective Activity of Water Extracts from Chaga Medicinal Mushroom, Inonotus obliquus (Higher Basidiomycetes) Against Tert-Butyl Hydroperoxide-Induced Oxidative Liver Injury in Primary Cultured Rat Hepatocytes.

Int J Med Mushrooms. 2015;17(11):1069-76.

We examined the hepatoprotective activity of Inonotus obliquus water extract (IO-W) against tert-butyl hydroperoxide (t-BHP)-induced oxidative liver injury in the primary cultured rat hepatocyte. The 50% radical scavenging concentrations (SC50s) of IO-W for radical-scavenging activity against 2,2'-azino-bis-(3-ethylbenzothi- azoline-6-sulfonic acid) (ABTS) and 1,1-diphenyl-2-picryl-hydrazyl (DPPH) were 5.19 mg/mL and 0.39 mg/mL, respectively. IO-W pretreatment to the primary cultured hepatocytes significantly ($p<0.05$) protected the cells from t-BHP-induced cytotoxic injury even at a low concentration of IO-W (10 µg/mL). The cellular leakage of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH), as well as malondialdehyde (MDA) formation caused by t-BHP were significantly ($p<0.05$) suppressed by IO-W pretreatment (>100 µg/ mL). In conclusion, this study demonstrates that IO-W exhibited hepatoprotective activity against t-BHP-induced oxidative liver injury in the primary cultured hepatocyte probably via its abilities of quenching free radicals, inhibiting the leakage of ALT, AST, and LDH, and decreasing MDA formation.

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METABOLIC HEALTH

Structure Characterization and Hypoglycaemic Activities of Two Polysaccharides from Inonotus obliquus.

Molecules. 2018 Aug 4; 23(8). pii: E1948. doi: 10.3390/molecules23081948.

In the present study, two polysaccharides (HIOP1-S and HIOP2-S) were isolated and purified from Inonotus obliquus using DEAE-52 cellulose and Sephadex G-100 column chromatography. The structural characterization and in vitro and in vivo hypoglycaemic activities of these molecules were investigated. HPLC analysis HIOP1-S was a heterpolysaccharide with glucose and galactose as the main compontent monosaccharides (50.247%, molar percentages). However, HIOP2-S was a heterpolysaccharide with glucose as the main monosaccharide (49.881%, molar percentages). The average molecular weights of HIOP1-S and HIOP2-S were 13.6 KDa and 15.2 KDa, respectively. The β-type glycosidic bond in HIOP1-S and HIOP2-S was determined using infrared analysis. ^{1}H-NMR spectra indicated that HIOP2-S contains the β-configuration glycosidic bond, and the glycoside bonds of HIOP1-S are both α-type and β-type. The ultraviolet scanning showed that both HIOP1-S and HIOP2-S contained a certain amount of binding protein. Congo red test showed that HIOP1-S and HIOP2-S could form a regular ordered triple helix structure in the neutral and weakly alkaline range. HIOP1-S and HIOP2-S showed strong α-glucosidase inhibitory activities and increased the glucose consumption of HepG2 cells. In addition, Streptozotocin (STZ)-induced hyperglycaemic mice were used to evaluate the antihyperglycaemic effects of HIOP1-S and HIOP2-S in vivo. The results showed that HIOP2-S had antihyperglycaemic effects. Taken together, these results suggest that HIOP1-S and HIOP2-S have potential anti-diabetic effects.

Effects of polysaccharides from Inonotus obliquus and its chromium (III) complex on advanced glycation end-products formation, α-amylase, α-glucosidase activity and H2O2-induced oxidative damage in hepatic L02 cells.

Food Chem Toxicol. 2018 Jun; 116(Pt B):335-345. doi: 10.1016/j.fct.2018.04.047. Epub 2018 Apr 22.

In the present study, the antioxidant activity, anti-glycation activity, α-amylase, α-glucosidase inhibitory activity of polysaccharides from Inonotus obliquus (UIOPS) and its chromium (III) complex (UIOPC) were investigated. Their protective effects against H2O2-induced oxidative damages in hepatic L02 cells were also assessed. Results demonstrated that UIOPC and UIOPS exhibited remarkable DPPH scavenging activity, ferric reducing power and hemolysis inhibitory activity. UIOPC also showed significant inhibitory capacity on α-amylase and α-glucosidase than UIOPS ($P < 0.05$), suggesting a good regulation of the postprandial hyperglycemia. Three phases of advanced glycation end products (AGEs) formation were effectively inhibited by UIOPC and UIOPS. Moreover, pretreatment with UIOPC and UIOPS markedly attenuated the oxidative damage induced by H2O2 in hepatic L02 cells via enhancing the cell viability, inhibiting the morphology alteration and maintaining the integrity of mitochondria. These results indicated that the anti-diabetic mechanism of UIOPC might involve in the homoeostasis of blood glucose and the recovery of endogenous antioxidant system. The elucidation of the potential anti-diabetic mechanism will facilitate the further study and application of the polysaccharides-metal complex in the functional food industry.

Effects of simulated gastrointestinal digestion in vitro on the chemical properties, antioxidant activity, α-amylase and α-glucosidase inhibitory activity of polysaccharides from Inonotus obliquus.

Food Res Int. 2018 Jan; 103:280-288. doi: 10.1016/j.foodres.2017.10.058. Epub 2017 Oct 31.

This study was aimed to investigate the impacts of gastrointestinal digestion in vitro on the physicochemical properties and the biological activities of polysaccharides from Inonotus obliquus (UIOPS-1). Results showed that the monosaccharides composition, structure and conformation of polysaccharides were remarkably altered, and the molecular weight (Mw) of UIOPS-

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1 was steadily decreased from 105.02kDa to 88.97kDa and 36.74kDa after simulated digestion. The reducing ends were increased and the free monosaccharides including d-glucose, d-galactose and d-xylose were released suggested that the degradation of UIOPS-1 was caused by disrupting the aggregates and glycosidic bonds. The antioxidant activities of UIOPS-1 were significantly increased after gastric digestion (P<0.05), however, they were decreased after intestinal digestion. The α-amylase and α-glucosidase inhibitory activities of UIOPS-1 were significantly increased after digestion indicating a good control of postprandial hyperglycemia (P<0.001). The results indicated that UIOPS-1 still exhibited antioxidant and antihyperglycemic potential after gastrointestinal digestion, which could be considered as a promising candidate for functional foods.

Anti-diabetic effects of Inonotus obliquus polysaccharides in streptozotocin-induced type 2 diabetic mice and potential mechanism via PI3K-Akt signal pathway.

Biomed Pharmacother. 2017 Nov; 95:1669-1677. doi: 10.1016/j.biopha.2017.09.104. Epub 2017 Oct 6.

Polysaccharides are the main components of mushroom Inonotus obliquus (I. obliquus) with antihyperglycemic activities. This study was aimed to investigate the anti-diabetic effects and the potential mechanism of I. obliquus polysaccharides (IOPS) in high fat diet and STZ-induced type 2 diabetic mice. Results showed that oral administration of IOPS (900mg/kg) could significantly restore the body and fat mass weight, reduce fasting blood glucose levels, improve glucose tolerance ability, increase hepatic glycogen level and ameliorate insulin resistance compared to those of the control diabetic mice (P<0.01). IOPS (900mg/kg) could enhance the cholesterol transportation in the liver, which was in coincidence with the increased HDL-C levels and decreased TC, TG and LDL-C levels.Treatment of IOPS could significantly improve the antioxidant activities of liver (P<0.05) and alleviate the STZ-lesioned organ tissues (liver, kidney, and pancreas). Further, protein expressions of PI3K-p85, p-Akt (ser473), GLUT4 were up-regulated after IOPS treatment, indicating that the antihyperglycemic mechanism of IOPS might involve in activating PI3K and Akt phosphorylation as well as the translocation of GLUT4 in diabetic mice. The results suggested that IOPS might be a promising functional food or drug candidate for diabetes treatment.

Antidiabetic activities of polysaccharides separated from Inonotus obliquus via the modulation of oxidative stress in mice with streptozotocin-induced diabetes.

PLoS One. 2017 Jun 29;12(6):e0180476. doi: 10.1371/journal.pone.0180476. eCollection 2017.

This study evaluated the effects of Inonotus obliquus polysaccharides (IOs) on diabetes and other underlying mechanisms related to inflammatory factors and oxidative stress in a mouse model of streptozotocin (STZ)-induced diabetes. Four weeks administration of metformin (120 mg/kg) and IO1-4 (50%-80% alcohol precipitation), or IO5 (total 80% alcohol precipitation) at doses of 50 mg/kg reverses the abnormal changes of bodyweights and fasting blood glucose levels of diabetic mice. IOs significantly increased the insulin and pyruvate kinase levels in serum, and improved the synthesis of glycogen, especially for IO5. IOs restored the disturbed serum levels of superoxide dismutase, catalase, glutathione peroxidase, and malondialdehyde. The down-regulation of interleukin-2 receptor, matrix metalloproteinase-9, and the enhancement of interleukin-2 in serum of diabetic mice were significantly attenuated by IOs. Histologic and morphology examinations showed that IOs repaired the damage on kidney tissues, inhibited inflammatory infiltrate and extracellular matrix deposit injuries in diabetic mice. Compared with untreated diabetic mice, IOs decreased the expression of phosphor-NF-κB in the kidneys. These results show that IOs treatment attenuated diabetic and renal injure in STZ-induced diabetic mice, possibly through the modulation of oxidative stress and inflammatory factors. These results provide valuable evidences to support the use of I. obliquus as a hypoglycemic functional food and/or medicine.

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Effects of the herb mixture, DTS20, on oxidative stress and plasma alcoholic metabolites after alcohol consumption in healthy young men.

Integr Med Res. 2016 Dec;5(4):309-316. doi: 10.1016/j.imr.2015.10.001. Epub 2015 Oct 13.

BACKGROUND: This study was designed to investigate the effect of a herbal mixture extract (DTS20) on the attenuation of oxidative stress and hangover after alcohol consumption in healthy volunteers.

METHODS: DTS20 consists of Viscum album L. (40%), Lycium chinense L. (30%), Inonotus obliquus (20%), and Acanthopanax senticosus H. (10%). We recruited healthy, nonsmoking, adult men volunteers aged between 21 years and 30 years to participate in a crossover trial. Twenty participants received either one package of placebo with 200 mL water or DTS20 with 200 mL water. Thirty minutes later, the volunteers ingested one bottle of Soju, which is a commercially available liquor (19% alcohol in 360 mL).

RESULTS: Volunteers received the opposite treatment after a 1-week washout period. DTS20 is mainly composed of sugars (564.5 mg/g) and polyphenol (28.2 mg/g). Alcohol levels in the DTS20 group were significantly lower than the control group at 2 hours after drinking Soju ($p < 0.05$). Acetaldehyde levels in the DTS20 group tended to be lower than the control group at 2 hours after drinking Soju, but was not significantly different. The antioxidant activity level was also significantly different between the control and DTS20 group 2 hours after drinking Soju ($p < 0.05$). No differences in plasma alanine transaminase or aspartate transaminase levels were observed between plasma levels before drinking and 2 hours after drinking Soju in the control group.

CONCLUSION: It was concluded that DTS20 reduced oxidative stress and hangover by mitigating plasma alcohol concentrations and elevating antioxidative activity in healthy male adults.

Anti-diabetic effects of Inonotus obliquus polysaccharides-chromium (III) complex in type 2 diabetic mice and its sub-acute toxicity evaluation in normal mice.

Food Chem Toxicol. 2017 Oct;108(Pt B):498-509. doi: 10.1016/j.fct.2017.01.007. Epub 2017 Jan 11.

Polysaccharides are important bioactive ingredients from Inonotus obliquus. This study aimed to synthesize and characterize a novel I. obliquus polysaccharides-chromium (III) complex (UIOPC) and investigate the anti-diabetic effects in streptozotocin (STZ) induced type 2 diabetes mellitus (T2DM) mice and sub-acute toxicity in normal mice. The molecular weight of UIOPC was about 11.5×10^4 Da with the chromium content was 13.01% and the chromium was linked with polysaccharides through coordination bond. After treatment of UIOPC for four weeks, the body weight, fasting blood glucose (FBG) levels, plasma insulin levels of the diabetic mice were significantly reduced when compared with those of the diabetic mice ($p < 0.05$). The results on serum profiles and antioxidant enzymes activities revealed that UIOPC had a positive effect on hypoglycemic and antioxidant ability. Histopathology results showed that UIOPC could effectively alleviate the STZ-lesioned tissues in diabetic mice. Furthermore, high dose administration of UIOPC had no obviously influence on serum profiles levels and antioxidant ability of the normal mice and the organ tissues maintained organized and integrity in the sub-acute toxicity study. These results suggested that UIOPC might be a good candidate for the functional food or pharmaceuticals in the treatment of T2DM.

Effect of Inonotus Obliquus Polysaccharides on physical fatigue in mice.

J Tradit Chin Med. 2015 Aug;35(4):468-72.

OBJECTIVE: To evaluate the potential beneficial effects of Inonotus obliquus polysaccharides (IOP) on the alleviation of physical fatigue in mice.

METHODS: Sixty-four male mice were randomly divided into four groups (n = 16 per group). Mice were orally administered IOP for a period of 14 days at 0, 100, 200 and 300 mg/kg/d, and were assigned to the control, IOP-100, IOP-200, and IOP-300 groups,

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respectively by the random number table method. Mice in the control group received an oral administration of sterile distilled water. A forced swimming test was performed for 8 mice per group at one hour after the last treatment. The other 8 mice in each group swam for 30 min. Blood, liver and muscle samples were taken after resting for 30 min. Levels of blood urea nitrogen and lactate, as well as glycogen contents of the liver and muscle were measured. Morphology of liver was observed by light microscopy.

RESULTS: IOP extended the swimming time of mice, and increased the glycogen content of liver and muscle, but decreased blood lactic acid and serum urea nitrogen levels, IOP had no toxic effects on major organs such as the liver as assessed by histopathological examinations.

CONCLUSION: IOP might be a potential anti-fatigue pharmacological agent.

Ameliorating effects of Inonotus obliquus on high fat diet-induced obese rats.

Acta Biochim Biophys Sin (Shanghai). 2015 Sep; 47(9):755-7. doi: 10.1093/abbs/gmv073. Epub 2015 Aug 4.

No abstract available for this publication.

Terpenoids with alpha-glucosidase inhibitory activity from the submerged culture of Inonotus obliquus.

Phytochemistry. 2014 Dec; 108:171-6. doi: 10.1016/j.phytochem.2014.09.022. Epub 2014 Oct 18.

Lanostane-type triterpenoids, inotolactones A and B, a drimane-type sesquiterpenoid, inotolactone C, and five known terpenoids 6β-hydroxy-trans-dihydroconfertifolin, inotodiol, 3β,22-dihydroxyanosta-7,9(11),24-triene, 3β-hydroxycinnamolide, and 17-hydroxy-ent-atisan-19-oic acid, were isolated from the submerged culture of chaga mushroom, Inonotus obliquus. Their structures were characterized by spectroscopic methods, including MS and NMR (1D and 2D) spectroscopic techniques. Inotolactones A and B, examples of lanostane-type triterpenoids bearing α,β-dimethyl, α,β-unsaturated δ-lactone side chains, exhibited more potent alpha-glucosidase inhibitory activities than the positive control acarbose. This finding might be related to the anti-hyperglycemic properties of the fungus and to its popular role as a diabetes treatment. In addition, a drimane-type sesquiterpenoid and an atisane-type diterpenoid were isolated from I. obliquus.

Protective Effect of Polysaccharides from Inonotus obliquus on Streptozotocin-Induced Diabetic Symptoms and Their Potential Mechanisms in Rats.

Evid Based Complement Alternat Med. 2014; 2014:841496. doi: 10.1155/2014/841496. Epub 2014 Jun 30.

The present study aimed to evaluate the therapeutic effects of polysaccharides from Inonotus obliquus (PIO) on streptozotocin-(STZ-) induced diabetic symptoms and their potential mechanisms. The effect of PIO on body weight, blood glucose, damaged pancreatic β-cells, oxidative stresses, proinflammatory cytokines, and glucose metabolizing enzymes in liver was studied. The results show that administration of PIO can restore abnormal oxidative indices near normal levels. The STZ-damaged pancreatic β-cells of the rats were partly recovered gradually after the mice were administered with PIO 6 weeks later. Therefore, we may assume that PIO is effective in the protection of STZ-induced diabetic rats and PIO may be of use as antihyperglycemic agent.

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Insulin-sensitizing and beneficial lipid-metabolic effects of the water-soluble melanin complex extracted from Inonotus obliquus.

Phytother Res. 2014 Sep; 28(9):1320-8. doi: 10.1002/ptr.5131. Epub 2014 Feb 24.

Inonotus obliquus has been traditionally used for treatment of metabolic diseases; however, the mechanism remains to be elucidated. In this study, we found that the water-soluble melanin complex extracted from I. obliquus improved insulin sensitivity and reduced adiposity in high fat (HF)-fed obese mice. When the melanin complex was treated to 3T3-L1 adipocytes, insulin-stimulated glucose uptake was increased significantly, and its phosphoinositide 3-kinase-dependent action was proven with wortmannin treatment. Additionally, dose-dependent increases in Akt phosphorylation and glucose transporter 4 translocation into the plasma membrane were observed in melanin complex-treated cells. Adiponectin gene expression in 3T3-L1 cells incubated with melanin complex increased which was corroborated by increased AMP-activated protein kinase phosphorylation in HepG2 and C2C12 cells treated with conditioned media from the 3T3-L1 culture. Melanin complex-treated 3T3-L1 cells showed no significant change in expression of several lipogenic genes, whereas enhanced expressions of fatty acid oxidative genes were observed. Similarly, the epididymal adipose tissue of melanin complex-treated HF-fed mice had higher expression of fatty acid oxidative genes without significant change in lipogenic gene expression. Together, these results suggest that the water-soluble melanin complex of I. obliquus exerts antihyperglycemic and beneficial lipid-metabolic effects, making it a candidate for promising antidiabetic agent.

Comparison of hypoglycemic activity of fermented mushroom of Inonotus obliquus rich in vanadium and wild-growing I. obliquus.

Biol Trace Elem Res. 2011 Dec; 144(1-3):1351-7. doi: 10.1007/s12011-011-9043-8. Epub 2011 Apr 5.

The effects of vanadium-enriched and wild Inonotus obliquus were tested on hyperglycemic mice. The vanadium content of the culture medium was 0.6%, reaching a concentration of 3.0 mg/g in the cultured mushroom while in the wild variety is 1/100 of that amount. The toxicity of vanadium at the 3.0 mg/g level is negligible, but its anti-diabetic effects are significantly different to those of the wild variety ($p < 0.05$). Due to its high bioavailability and low toxicity, vanadium-enriched I. obliquus could be used as a means of vanadium supplementation, with expectation of obtaining higher bioavailability and lower toxicity in animals.

Extract of Chaga mushroom (Inonotus obliquus) stimulates 3T3-L1 adipocyte differentiation.

Phytother Res. 2010 Nov; 24(11):1592-9. doi: 10.1002/ptr.3180.

Chaga mushroom (Inonotus obliquus) has long been used as a folk medicine due to its numerous biological functions such as antibacterial, antiallergic, antiinflammatory and antioxidative activities. In the present study, it was found that the I. obliquus hot water extract (IOWE) activated adipogenesis of 3T3-L1 preadipocytes. Even in the absence of adipogenic stimuli by insulin, the IOWE strongly induced adipogenesis of 3T3-L1 preadipocytes. The major constituent of IOWE was glucose-rich polysaccharides with a molecular mass of 149 kDa. IOWE enhanced the differentiation of 3T3-L1 preadipocytes, increasing TG (triacylglycerol) accumulation that is critical for acquisition of the adipocyte phenotype, in a dose-dependent manner. IOWE stimulated gene expression of C/EBPα (CCAAT/enhancer-binding protein α) and PPARγ (peroxisome proliferator-activated receptors γ) during adipocyte differentiation, and induced the expression of PPARγ target genes such as aP2 (adipocyte protein 2), LPL (lipoprotein lipase) and CD36 (fatty acid translocase). Immunoblot analysis revealed that IOWE increased the expression of adipogenic makers such as PPARγ and GLUT4 (glucose transporter 4). The luciferase reporter assay demonstrated that IOWE did not exhibit PPARγ ligand activity. Although these results require further investigation, the ability of natural mushroom product to increase PPARγ transcriptional activities may be expected to be therapeutic targets for dyslipidemia and type 2 diabetes.

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Phytochemical characteristics and hypoglycaemic activity of fraction from mushroom Inonotus obliquus.

J Sci Food Agric. 2010 Jan 30; 90(2):276-80. doi: 10.1002/jsfa.3809.

BACKGROUND: Inonotus obliquus is a medicinal mushroom that has been used as an effective agent to treat various diseases such as diabetes, tuberculosis and cancer. In order to elucidate the active fraction and its constituents, the effects of ethyl acetate fraction from I. obliquus (EAFI) on hyperglycaemia were investigated and the main constituents of EAFI were isolated and identified.

RESULTS: EAFI treatment led to a significant decrease in blood glucose level ($P < 0.05$) in alloxan-induced diabetic mice. It significantly decreased the total cholesterol level in serum, increased glutathione peroxidase activity and improved the growth physiological characteristics. In addition, EAFI treatment decreased the levels of triglyceride and malondialdehyde and increased the high-density lipoprotein cholesterol level in serum and the hepatic glycogen level in liver of diabetic mice. Five compounds were isolated from EAFI and identified as lanosterol (1), 3beta-hydroxy-lanosta-8,24-diene-21-al (2), inotodiol (3), ergosterol peroxide (4) and trametenolic acid (5) by spectral methods. Inotodiol and trametenolic acid were found to have an inhibitory effect on alpha-amylase activity and a scavenging effect on 1,1-diphenyl-2-picrylhydrazyl radicals.

CONCLUSION: EAFI showed significant antihyperglycaemic and antilipidperoxidative effects in alloxan-induced diabetic mice. Terpenoid and sterol compounds appeared to be the major active constituents of I. obliquus.

Beneficial effects of the ethanol extract from the dry matter of a culture broth of Inonotus obliquus in submerged culture on the antioxidant defence system and regeneration of pancreatic beta-cells in experimental diabetes in mice.

Nat Prod Res. 2010 Apr; 24(6):542-53. doi: 10.1080/14786410902751009.

The antihyperglycaemic and antilipidperoxidative effects of the ethanol extract from the dry matter of a culture broth (DMCB) of Inonotus obliquus were investigated in alloxan-induced diabetic mice and the possible mechanism of action was also discussed. In alloxan-induced diabetic mice, treatment with the ethanol extract from DMCB of I. obliquus (30 and 60 mg kg(-1) body weight (b.w.) for 21 days) showed a significant decrease in blood glucose level: the percentage reductions on the 7th day were 11.54 and 11.15%, respectively. However, feeding of this drug for three weeks produced reduction of 22.51 and 24.32%. Furthermore, the ethanol extract from the DMCB of I. obliquus treatment significantly decreased serum contents of free fatty acids, total cholesterol, triglycerides and low-density lipoprotein-cholesterol, whereas it effectively increased high-density lipoprotein-cholesterol, insulin levels and hepatic glycogen contents in livers of diabetic mice. Besides this, the ethanol extracts from the DMCB treatment significantly increased catalase, superoxide dismutase and glutathione peroxidase activities, except for decreasing the maleic dialdehyde level in diabetic mice. Histological morphology examination showed that the ethanol extract from the DMCB of I. obliquus restored the damage of pancreatic tissues in mice with diabetes mellitus. The results showed that the ethanol extract from the DMCB of I. obliquus possesses significant antihyperglycaemic, antilipidperoxidative and antioxidant effects in alloxan-induced diabetic mice.

Antihyperglycemic and antilipidperoxidative effects of dry matter of culture broth of Inonotus obliquus in submerged culture on normal and alloxan-diabetes mice.

J Ethnopharmacol. 2008 Jun 19; 118(1):7-13. doi: 10.1016/j.jep.2008.02.030. Epub 2008 Mar 4.

AIM OF THE STUDY: The antihyperglycemic and antilipidperoxidative effects of the dry matter of culture broth (DMCB) of Inonotus obliquus were investigated.

MATERIALS AND METHODS: The normal, glucose-induced hyperglycemic and alloxan-induced diabetic mice were used to evaluate the antihyperglycemic and antilipidperoxidative effects of the DMCB of Inonotus obliquus.

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RESULTS: Treatment with the DMCB (500 and 1000 mg/kg body weight) exhibited a mild hypoglycemic effect in normal mice, and failed to reduce the peak glucose levels after glucose administration. However, euglycemia was achieved in the DMCB of Inonotus obliquus (1000 mg/kg) and glibenclamide-treated mice after 120 min of glucose loading. In alloxan-induced diabetic mice, the DMCB (500 and 1000 mg/kg body weight for 21 days) showed a significant decrease in blood glucose level, the percentages reduction on the 7th day were 11.90 and 15.79%, respectively. However, feeding of this drug for 3 weeks produced reduction was 30.07 and 31.30%. Furthermore, the DMCB treatment significantly decreased serum contents of free fatty acid (FFA), total cholesterol (TC), triglyceride (TG) and low density lipoprotein-cholesterol (LDL-C), whereas effectively increased high density lipoprotein-cholesterol (HDL-C), insulin level and hepatic glycogen contents in liver on diabetic mice. Besides, the DMCB treatment significantly increased catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities except for decreasing maleic dialdehyde (MDA) level in diabetic mice. Histological morphology examination showed that the DMCB restored the damage of pancreas tissues in mice with diabetes mellitus.

CONCLUSIONS: The results showed that the DMCB of Inonotus obliquus possesses significant antihyperglycemic, antilipidperoxidative and antioxidant effects in alloxan-induced diabetic mice.

Isolation and characterization of a novel platelet aggregation inhibitory peptide from the medicinal mushroom, Inonotus obliquus.

Peptides. 2006 Jun; 27(6):1173-8. Epub 2005 Nov 11.

This study describes the extraction and characterization of a platelet aggregation inhibitory peptide from Inonotus obliquus. Ethanol extract from I. obliquus ASI 74006 mycelia showed the highest platelet aggregation inhibitory activity (81.2%). The maximum platelet aggregation inhibitory activity was found when the mycelia of I. obliquus ASI 74006 was extracted with ethanol at 80 degrees C for 12 h. The platelet aggregation inhibitor was purified by systematic solvent fractionation, ultrafiltration, Sephadex G-10 column chromatography, and reverse-phase HPLC. The purified platelet aggregation inhibitor is a novel tripeptide with a molecular mass of 365 Da, having a sequence of Trp-Gly-Cys. The purified platelet aggregation inhibitor also showed high platelet aggregation inhibitory activity in Institute of Cancer Research (ICR) mice.

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PHARMACOGNOSY

Review on Chaga Medicinal Mushroom, Inonotus obliquus (Higher Basidiomycetes): Realm of Medicinal Applications and Approaches on Estimating its Resource Potential.

Int J Med Mushrooms. 2015; 17(2):95-104.

This paper presents a review of the realm of medicinal applications of Inonotus obliquus raw materials, sterile conks I. obliquus, based on the bibliographies of chemical studies of the fungus. The experimental part of the paper is devoted to the presentation of methods of estimating the resource potential of this fungus based on data obtained in the comfort zone of ththis species. A new form, I. obliquus f. sterilis, is formally described.

Amelioration of scopolamine induced cognitive dysfunction and oxidative stress by Inonotus obliquus - a medicinal mushroom.

Food Funct. 2011 Jun; 2(6):320-7. doi: 10.1039/c1fo10037h. Epub 2011 Jun 6.

The present study was aimed to investigate the cognitive enhancing and anti-oxidant activities of Inonotus obliquus (Chaga) against scopolamine-induced experimental amnesia. Methanolic extract of Chaga (MEC) at 50 and 100 mg kg (-1) doses were administered orally for 7 days to amnesic mice. Learning and memory was assessed by passive avoidance task (PAT) and Morris water maze (MWM) test. Tacrine (THA, 10 mg kg (-1), orally (p.o)) used as a reference drug. To elucidate the mechanism of the cognitive enhancing activity of MEC, the activities of acetylcholinesterase (AChE), anti-oxidant enzymes, the levels of acetylcholine (ACh) and nitrite of mice brain homogenates were evaluated. MEC treatment for 7 days significantly improved the learning and memory as measured by PAT and MWM paradigms. Further, MEC significantly reduced the oxidative-nitritive stress, as evidenced by a decrease in malondialdehyde and nitrite levels and restored the glutathione and superoxide dismutase levels in a dose dependent manner. In addition, MEC treatment significantly decreased the AChE activity in both the salt and detergent-soluble fraction of brain homogenates. Further, treatment with MEC restored the levels of ACh as did THA. Thus, the significant cognitive enhancement observed in mice after MEC administration is closely related to higher brain anti-oxidant properties and inhibition of AChE activity. These findings stress the critical impact of Chaga, a medicinal mushroom, on the higher brain functions like learning and memory.

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SKIN HEALTH / COSMETICS

Inhibitory and Acceleratory Effects of Inonotus obliquus on Tyrosinase Activity and Melanin Formation in B16 Melanoma Cells.

Evid Based Complement Alternat Med. 2014; 2014:259836. doi: 10.1155/2014/259836. Epub 2014 Aug 13.

The aim of the present study is to preliminarily investigate the antimelanogenesis effect of Inonotus obliquus extracts by cell-free mushroom tyrosinase assay. It was found that petroleum ether and n-butanol extracts might contain unknown potential tyrosinase inhibitors, while its ethyl acetate extract might contain some unknown accelerators. Six compounds were isolated and their structures were identified by interpretation of NMR data and nicotinic acid was first discovered in Inonotus obliquus. In cells testing, betulin and trametenolic acid decreased tyrosinase activity and melanin content, while inotodiol and lanosterol significantly increased tyrosinase activity and melanin content, showing an AC50 of 9.74 and 8.43 μM, respectively. Nicotinie acid, 3β,22,25-trihydroxy-lanosta-8-ene, had a little or no effect on tyrosinase. Betulin exhibited a mode of noncompetitive inhibition with a K I = K IS of 0.4 μM on tyrosinase activity showing an IC50 of 5.13 μM and being more effective than kojic acid (6.43 μM), and trametenolic acid exhibited a mode of mixed inhibition with a K I of 0.9 μM, K IS of 0.5 μM, and an IC50 of 7.25 μM. We proposed betulin and trametenolic acid as a new candidate of potent tyrosinase inhibitors and inotodiol and lanosterol as accelerators that could be used as therapeutic agent.

Inonotus obliquus protects against oxidative stress-induced apoptosis and premature senescence.

Mol Cells. 2011 May; 31(5):423-9. doi: 10.1007/s10059-011-0256-7. Epub 2011 Feb 22.

In this study, we investigated the cytoprotective effects of Inonotus obliquus against oxidative stress-induced apoptosis and premature senescence. Pretreatment with I. obliquus scavenged intracellular ROS and prevented lipid peroxidation in hydrogen peroxide-treated human fibroblasts. As a result, I. obliquus exerted protective effects against hydrogen peroxide-induced apoptosis and premature senescence in human fibroblasts. In addition, I. obliquus suppressed UV-induced morphologic skin changes, such as skin thickening and wrinkle formation, in hairless mice in vivo and increased collagen synthesis through inhibition of MMP-1 and MMP-9 activities in hydrogen peroxide-treated human fibroblasts. Taken together, these results demonstrate that I. obliquus can prevent the aging process by attenuating oxidative stress in a model of stress-induced premature senescence.

Extract of Chaga mushroom (Inonotus obliquus) stimulates 3T3-L1 adipocyte differentiation.

Phytother Res. 2010 Nov; 24(11):1592-9. doi: 10.1002/ptr.3180.

Chaga mushroom (Inonotus obliquus) has long been used as a folk medicine due to its numerous biological functions such as antibacterial, antiallergic, antiinflammatory and antioxidative activities. In the present study, it was found that the I. obliquus hot water extract (IOWE) activated adipogenesis of 3T3-L1 preadipocytes. Even in the absence of adipogenic stimuli by insulin, the IOWE strongly induced adipogenesis of 3T3-L1 preadipocytes. The major constituent of IOWE was glucose-rich polysaccharides with a molecular mass of 149 kDa. IOWE enhanced the differentiation of 3T3-L1 preadipocytes, increasing TG (triacylglycerol) accumulation that is critical for acquisition of the adipocyte phenotype, in a dose-dependent manner. IOWE stimulated gene expression of C/EBPα (CCAAT/enhancer-binding protein α) and PPARγ (peroxisome proliferator-activated receptors γ) during adipocyte differentiation, and induced the expression of PPARγ target genes such as aP2 (adipocyte protein 2), LPL (lipoprotein lipase) and CD36 (fatty acid translocase). Immunoblot analysis revealed that IOWE increased the expression of adipogenic makers such as PPARγ and GLUT4 (glucose transporter 4). The luciferase reporter assay demonstrated that IOWE did not exhibit PPARγ ligand activity. Although these results require further investigation, the ability of natural mushroom

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product to increase PPARγ transcriptional activities may be expected to be therapeutic targets for dyslipidemia and type 2 diabetes.

New antioxidant polyphenols from the medicinal mushroom inonotus obliquus.

Bioorg Med Chem Lett. 2007 12/15; 17(24):6678-81.

The fruiting body of Inonotus obliquus, a medicinal mushroom called chaga, has been used as a traditional medicine for cancer treatment. Although this mushroom has been known to exhibit potent antioxidant activity, the mechanisms responsible for this activity remain unknown. In our investigation for free radical scavengers from the methanolic extract of this mushroom, inonoblins A (1), B (2), and C (3) were isolated along with the known compounds, phelligridins D (4), E (5), and G (6). Their structures were established by extensive spectroscopic analyses. These compounds exhibited significant scavenging activity against the ABTS radical cation and DPPH radical, and showed moderate activity against the superoxide radical anion.

Polysaccharides isolated from Phellinus gilvus inhibit melanoma growth in mice.

2005 Cancer Lett, 218, 43-52.

There is no information about the effect of polysaccharides from fungus, Phellinus gilvus (PG) on melanoma. The effect of PG on the proliferation and apoptosis of the B16F10 melanoma cell line was determined by a sulforhodamine B (SRB) and a sandwich enzyme-linked immunosorbent assay. The in vivo effect of PG on B16F10 melanoma cells allografted in athymic nude mice was investigated. PG decreased cell proliferation and increased cell apoptosis in a dose dependent manner in vitro. Also, PG significantly inhibits melanoma growth in mice. The PG anti-tumor effect in vivo was associated with a significant increase in the melanoma apoptosis rate. These findings support PG as a therapeutic agent against melanoma.

Novel natural approaches to anti-aging skin care.

Cosmetics and Toiletries Manufacture Worldwide, 2002: 11-15.

Novel natural approaches to anti-aging skin care, targeting the root causes of skin damage and texture loss, are presented. Four proprietary extracts, Boswellin® (a natural extract derived from Indian frankincense), Umbelliferin® (a composition derived from coriander seeds), Lupeol 80% (a multifunctional natural extract from Crataeva nurvula (Varuna) and CococinTM (a patent-pending composition derived from green coconut water) are highlighted. The use of these extracts in anti-aging skin care compositions is described.

The effects of radicals compared with UVB as initiating species for the induction of chronic cutaneous photodamage.

J Invest Dermatol. 1999 Jun; 112(6):933-8.

There is substantial evidence that ultraviolet radiation induces the formation of reactive oxygen species which are implicated as toxic intermediates in the pathogenesis of photoaging. The aim of this study was to determine whether repeated topical treatment with benzoyl peroxide, a source of free radicals, produced the same cutaneous effects as chronic ultraviolet B radiation. Three concentrations of benzoyl peroxide (0.1, 1.5, 5.0% wt/wt) and three cumulative fluences of ultraviolet B radiation (0.9, 2.2, 5.1 J per cm2) used alone and in all combinations along with appropriate controls. Female SKH1 (hr/hr) albino hairless mice were treated 5 d per wk for 12 wk. Extracellular matrix molecules and histologic parameters were assessed. Ultraviolet B radiation induced a fluence-dependent and time-dependent increase in skin-fold thickness. Fluence dependence was seen for

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epidermal thickness, sunburn cell numbers, dermal thickness, glycosaminoglycan content, mast cell numbers, and skin-fold thickness. Benzoyl peroxide treatment alone caused less marked increases in epidermal and dermal measures compared with ultraviolet B under the conditions used. A benzoyl peroxide concentration-dependent increase was only observed for elastin content, although the highest concentration of benzoyl peroxide increased epidermal thickness and glycosaminoglycan content. A synergistic interaction between ultraviolet B and benzoyl peroxide was not found. These results indicate that repeated administration of benzoyl peroxide produces skin changes in the hairless mouse that qualitatively resemble those produced by ultraviolet B and suggest that common mechanisms may be involved. In addition, any potential synergistic effect of ultraviolet B and benzoyl peroxide was below the level of detection limit.

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