

IMMUNOMODULATING EFFECTS OF CHAGA MUSHROOMS BIBLIOGRAPHY

Chaga mushroom is considered a natural Biological Response Modifier (BRM) mainly due to its high content of a class of polysaccharides known as Beta-D-Glucans and other key nutrients that help activate an array of immune cells, including lymphocytes, macrophages, and natural killer cells. These cells allow the body to suppress the formation of chronic health conditions like autoimmune disease, allergies, and cancer.

Chaga is a powerful potentiating and immune-enhancing “superfood” with vast potential for the maintenance of a healthy immune system with a vast array of potential benefits in the management of food and asthma allergies, atopic dermatitis, inflammation (including autoimmune inflammatory conditions such as rheumatoid arthritis), atherosclerosis, thrombosis and human immunodeficiency virus (HIV).

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

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

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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.

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

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

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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 $6.1 \times 10(4)$, $2.9 \times 10(4)$ and $3.5 \times 10(4)$ 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.

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

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

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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.

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

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

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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-1 β , 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 μ g/ml while exopolysaccharides and endopolysaccharides showed a much better effect on IL-1 β production at 30 μ g/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.

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PMID: 24867795 [Indexed for MEDLINE]

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

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

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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- κ B 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), 3 β -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.

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6. Int Immunopharmacol. 2013 Apr;15(4):666-70. doi: 10.1016/j.intimp.2013.03.015. Epub 2013 Mar 25.

Inhibitory effect of chaga mushroom extract on compound 48/80-induced anaphylactic shock and IgE production in mice.

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Chaga mushrooms (*Inonotus obliquus*) are hypothesised to exhibit general immune-potentiating, anti-inflammatory, and antitumor properties, but their anti-allergic activities are not fully understood. Therefore, this study investigated whether a chaga mushroom extract (C-HE) might have anti-allergic activity. This activity was assessed through the levels of the IgE Ab produced in

response to an allergen (OVA). The administration of C-HE prophylactically inhibited the systemic anaphylactic shock induced by compound 48/80 in mice. The oral administration of C-HE significantly reduced the total IgE levels in mice and slightly affected the production of IgG1. Furthermore, spleen cell cultures harvested from OVA-sensitized mice that had received C-HE orally showed a significant increase in Th1-derived responses (IFN- γ production). Therefore, our results suggest that the chaga mushroom extract may be used as an anti-allergic functional food.

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PMID: 23535020 [Indexed for MEDLINE]

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

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

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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, *Paralichthys 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.

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PMID: 22484608 [Indexed for MEDLINE]

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

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

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

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

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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- κ B, 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- κ B signaling pathways, suggesting that PFIO may potentially regulate the immune response.

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PMID: 21191814 [Indexed for MEDLINE]

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

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

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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/H₂O 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-1 β , TNF α 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-1 β , IL-6 and TNF α 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.

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PMID: 19577624 [Indexed for MEDLINE]

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

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

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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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PMID: 15970296 [Indexed for MEDLINE]

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

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

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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.

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