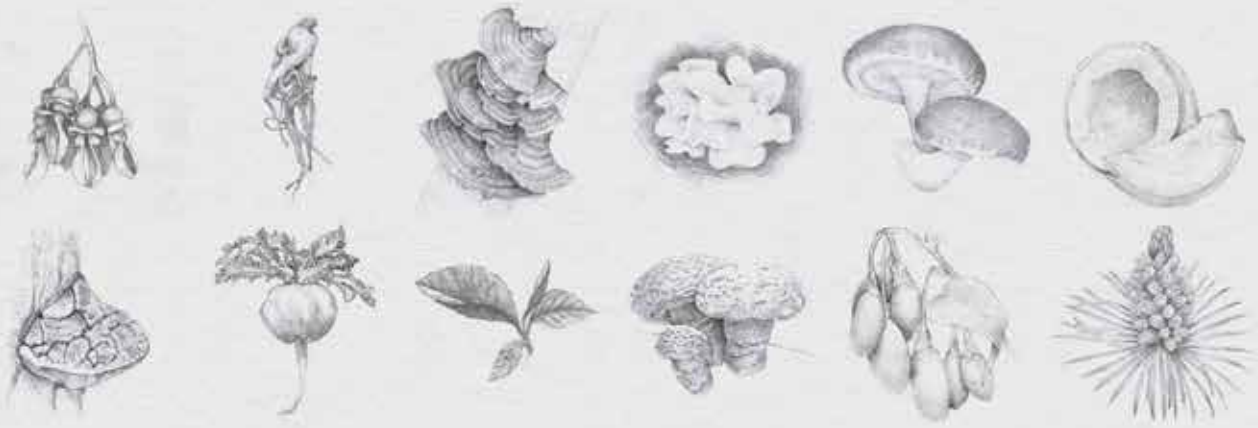
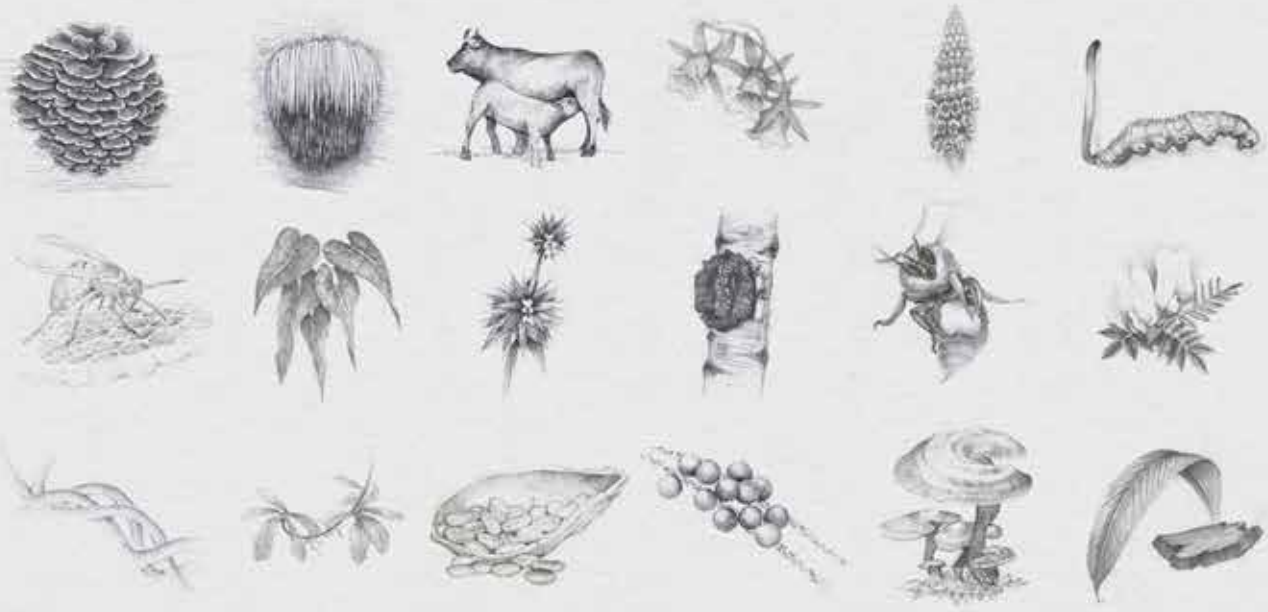


# FOUR SIGMA FOODS

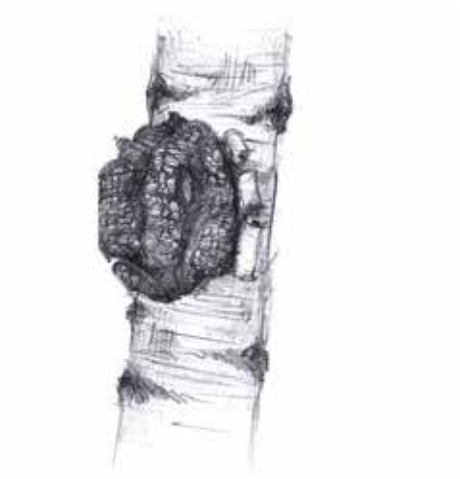
# SCIENCE

## CHAGA MUSHROOM



# CHAGA MUSHROOM

## *INONOTUS OBLIQUUS*



Mushrooms comprise a vast and yet largely untapped source of powerful new pharmaceutical products. In particular, and most importantly for modern medicine, they represent an unlimited source of polysaccharides with antitumor and immune-stimulating properties. Many, if not all, *Basidiomycetes* mushrooms contain biologically active polysaccharides in fruit bodies and cultured mycelium. [(1)]

The white rot fungus Chaga (*Inonotus obliquus*) belongs to the Hymenochaetaceae family of Basidiomycetes[(2)]. This fungus is usually found as a sterile conk (sclerotia) on Betula species, for example birch, in nature[(3)]. Chaga mushrooms have been used in folk medicine for instance treating cancer in Russia, Western Siberia, Asia and North America. It has been shown that Chaga mushrooms contain many steroids and phenolic compounds and that Chaga mushrooms also show various biological activities, including anti-bacterial, hepato-protective and antitumor properties. Several anti-cancer drugs or cancer treatment compounds have been found in natural products. [(2)(3).]

This short summary of Inonotus obliquus will briefly discuss some scientific results. Among those are anti-mutagenic and anti-cancer properties, activity to scavenge free radicals and immune-stimulating effects. Moreover some differences between sclerotial and mycelial *Inonotus obliquus* will be named. It will also be explained how does the structure and linkage of the polysaccharide affect how good antitumor or immune-stimulating activity the fungus will have.

## DIFFERENCES BETWEEN SCLEROTIAL AND MYCELIAL INONOTUS OBLIQUUS

Many medicinal mushrooms' polysaccharides are being commercially produced by submerged mycelium culture because of a high productivity compared to production from fruit bodies. Currently, there is a great need for mushroom polysaccharides. However, since the productivity of sclerotia is generally very low, submerged mycelium cultures offer a promising alternative method. Although mycelial fermentations of medicinal mushrooms have been often reported, only few have been using Chaga mushroom. There are many difficulties in large scale fermentations for production of mycelia-derived polysaccharides, such as a high contamination risk, a low growth rate, and sensitivity to environmental factors.[3] Moreover there have been reports showing that there is a large difference in the sugar content of polysaccharides from the mycelia and sclerotia of *Inonotus obliquus*. This affects the polysaccharides and in the other hand the immune-stimulating effects.[1]

**The sclerotial endo-polysaccharide exhibits anti-cancer effects directly through inhibition of cancer cells, whereas the mycelial variety produces an effect indirectly through activation of immune cells.** The polysaccharide productivity of sclerotia is very low compared to the productivity of mycelia. Therefore, the mycelial submerged culture method can be a good alternative for production of immune-stimulating polysaccharides. Sclerotia extracts of *Inonotus obliquus* are known to inhibit the growth and protein synthesis of tumor cells. However, it has been reported that polysaccharides from fungal sclerotia, which are known to include hetero-polysaccharide and homoglycan, showed strong antitumor effects while polysaccharides from cultured mycelia did not.[3]

## ANTI-MUTAGENIC AND ANTI-CANCER PROPERTIES OF INONOTUS OBLIQUUS

Cancer is a formidable problem for people. The great majority of chemical compounds, which have been identified as cytotoxic to cancer cells, are also toxic to normal cells. Therefore, it is very important to investigate novel antitumor substances with little toxicity to host. Recently, polysaccharides extracted from plants, fungi, algae and animals have been proven to have fewer side effects and possess a wide range of biological functions such as antitumor, antioxidant, immunomodulation and anti-tussive properties.[4] The polysaccharides from mushrooms have been proven not to attack cancer cells directly, but to produce their antitumor effects by activating different immune responses in the host. The antitumor action of polysaccharides requires an intact T-cell component; their activity is mediated through a thymus-dependent immune mechanism.[1]

Kim *et al.* [5] did a trial were they implanted melanoma cells into mice. During nine to ten days the mice were treated with intraperitoneal endo-polysaccharide, which is a special activator of B cells and macrophages, from *Inonotus obliquus* or fed orally with the same, but a different amount. Life threatening toxic effect was not detected in any group of the mice administrated intraperitoneally and orally with endo-polysaccharide. Mice that survived for 60 days did not show any tumor growth. The intraperitoneal treatment showed better effect and in most cases, intraperitoneal administration was more rapid and effective than oral. These results indicate that the endo-polysaccharide has a significant inhibitory effect on growth of melanoma tumor cells in the mice. **The in vivo antitumor effect of endo-polysaccharide is probably related to the activation of macrophages rather than to direct cytotoxicity against tumor cells.** It is believed that endo-polysaccharide can activate peritoneal macrophages and induce the production of host defense molecules, such as nitric oxide and cytokines. Nitrite has been identified as the major effective molecule involved in destruction of tumor cells by activated macrophages. **The nitrite production of purified endo-polysaccharide markedly increases compared to crude endo-polysaccharide, indicating that the purified endo-polysaccharides enhance phagocytosis and nitric oxide production in macrophages, resulting in immune-stimulating activity.** Thus, the anti-cancer effect of endo-polysaccharide in tumor-bearing mice is probably related to immune-stimulation.[5]

It has earlier been found that *Inonotus obliquus* extract bears antitumorigenic and anti-mutagenic properties. Ham et al. showed that the **pure compounds (subfractions) isolated from the ethyl acetate fraction of** *Inonotus obliquus* **relatively strongly inhibit the mutagenic activity of both directly and indirectly acting mutagens in a dose-dependent manner.** This suggests that these subfractions can protect the DNA or RNA in cells from mutagens. Alternatively, with regard to the indirectly acting mutagens, the subfractions may help inactivate these mutagenic precursors by hindering their transformation into carcinogens. Subfractions of *Inonotus obliquus* extracts possess a strong anti-mutagenic effect and may be useful as an ingredient in functional anti-cancer food.[2]

## IMMUNE-STIMULATING EFFECTS

The immune system plays an important role in antitumor defense. Splenocyte proliferation is a crucial event in the activation cascade of both cellular and humoral immune responses. T and B lymphocytes are two important classes of immunologically active cells. The former is mainly responsible for cellular immunity, and the latter is the only cell capable of producing antibodies. Macrophage is the most important professional phagocyte and it plays an essential and pivotal role in host defense against any type of invading cells including tumor cells. TNF-α plays an important role in tumoricidal and immune response and tumor cell elimination is known to be mediated in part by TNF-α. TNF-α can be produced by activated peritoneal macrophages. Some investigators have reported that the polysaccharides isolated from plants or fungi are immune-activators. The immune system becomes activated by the polysaccharides via stimulation of the T cells, B cells, natural killer (NK) cells and macrophages.[4][1]

Fan et al. found that in vivo water-soluble polysaccharide from *Inonotus obliquus* could significantly enhance the immune response of tumor-bearing mice. However, in vivo

water-soluble polysaccharide did not only show antitumor activity, but it could also enhance the lymphocyte proliferation and improve the ability of ConA-induced lymphocyte, which activates the T cells, proliferation. At the same time, water-soluble polysaccharide could mediate phagocytosis and increase the production of TNF-α compared with control group. The results indicated that the **water-soluble polysaccharide may indirectly play the role of antitumor activity through improving immunologic function** of tumor-bearing mice.[4]

*Photos: Charles de Martigny/ Flickr.com*

Lipopolysaccharide (LPS) is a part of the cell membrane in gram-negative bacteria, where they act as endotoxins. When LPS reach the human body or other animals, it activates the immune system. Some reports have shown that **the immune-stimulating activities of endo-polysaccharide in** *Inonotus obliquus* **were similar to those of LPS.** For example they both activate B cells and macrophages but not T cells. There is a difference in the action mechanisms of *Inonotus obliquus* polysaccharide and LPS. Mycelial endo-polysaccharide does not have a lipid-A moiety of LPS and seems to have other receptors that LPS does not have.[3]

## FREE RADICAL SCAVENGING ACTIVITY

In general, compounds that strongly inhibit the mutagenicity of indirectly and directly acting mutagens also have a high free radical scavenging activity[2]. Mushrooms usually contain a wide variety of free radical scavenging molecules, such as polysaccharide and polyphenols[6].

Ham *et al.*[2] determined the antioxidative activity of the Inonotus obliquus subfractions by using DPPH, which is a stable free radical that has been widely used as a substrate to evaluate the antioxidative activity of samples. This and earlier studies show that different kind of extracts and fractions of *Inonotus obliquus* and its insoluble polysaccharides have DPPH radical-scavenging activity. Lee *et al.* [7] did a similar study with Ham, where they found six major polyphenols antioxidants, three new inonoblins and three known phelligridings. The antioxidant activities of the polyphenols were evaluated by measuring free radical scavenging activity using three different assays: the DPPH radical scavenging activity assay, the ABTS radical scavenging activity assay, and the superoxide radical anion scavenging activity assay. The polyphenols found had significant scavenging activity against the ABTS radical cation and DPPH radical, and showed moderate activity against the superoxide radical anion. Lee et al. also compared the compounds from Inonotus obliquus with synthetic antioxidants such as butylated hydroxyanisole (BHA) and with trolox (a water-soluble derivative of vitamin E) and caffeic acid, which are well-known antioxidants and used as controls here. All polyphenols isolated exhibited higher ABTS cation radical scavenging activity than trolox and caffeic acid and some of the compounds showed better results than the synthetic BHA. Most of the compounds were less active than caffeic acid, tolux and BHA in the test of DPPH radical scavenging effect.

**Moreover polysaccharides and polyphenolic extracts are even compounds like triterpenoids and steroids having antioxidant activity.** Of these substances the polyphenolic extract have the strongest antioxidant activity, with the ability to scavenge free radicals by single-electron transfer and with a strong protective activity against hydrogen peroxide, H2O2, a strong oxidizer. This means that **polyphenols of** *Inonotus obliquus* **can protect cells against oxidative stress.** While Lee et al.[7] showed that polyphenols have moderate activity against the superoxide radical did Cui et al.[6] show that polyphenols are able to efficiently scavenge superoxide radicals.

Cui et al.[6] also did a study with an extract, containing triterpenoids and steroids including lanosterol, inotodiol, trametenolic acid and ergosterol peroxide that exhibited a relatively strong antioxidant effect. These results suggest that the triterpenoids and steroids in the extract may account for the free radical scavenging effect of *Inonotus obliquus*. The superoxide radical scavenging activity of the *Inonotus obliquus* extracts was generally quite high. However, the triterpenoids and steroids extract did not show any protective effect against hydrogen peroxide-induced oxidative stress, although they showed some free radical scavenging ability. These results suggest that hydrogen peroxide-induced cell death is related not only to free radicals, but also to unresolved signaling pathways[6].

## STRUCTURES

Polysaccharides with an antitumor activity differ greatly in their chemical compositions, configurations, and physical properties. Antitumor activity is exhibited by a wide range of glycans extending from homopolymers to highly complex heteropolymers. Differences in activity can be correlated with solubility in water, size of the molecules, branching rate, and form. Although it is difficult to correlate polysaccharide structure with antitumor activity, some relationships can be inferred. **The polysaccharide linkage type is another important factor for antitumor or immune-stimulating activity.** For instance, a triple-helical tertiary conformation of medicinal mushroom β(1→3)-glucans is known to be important for their immune-stimulating activity.[1]

**Even the molecular mass is said to be closely related to the biological activities** in medicinal mushrooms. In general, high molecular mass β-glucans appear to be more active than low molecular mass varieties. However, there have been no reports regarding a relationship of the molecular mass with biological activity in polysaccharides from the sclerotia and mycelia of *Inonotus obliquus*. According to a study of Kim et al.[3] the results show that most water-soluble endopolysaccharides smaller than 50 kDa had low activities. The 1100 kDa endo-polysaccharide seemed to be responsible for the high activities of polysaccharides from *Inonotus obliquus* mycelia.

It has also been shown that the polysaccharide activity is probably closely related to cell age.[3]

# CONCLUSIONS

*CHAGA MUSHROOM*

The white rot fungus Chaga *(Inonotus obliquus)* is usually found as a sterile conk (sclerotia) on birch trees. *Inonotus obliquus* has been used in folk medicine for a long time, for instance to treat cancer in areas like Russia and Western Siberia. From producing more polysaccharide of Inonotus obliquus after submerged mycelium has been cultivated, there are differences between the sclerotial and the mycelial fungus. For instance the sclerotial endo-polysaccharide exhibits anti-cancer effects directly through inhibition of cancer cells, whereas the mycelial variety produces an effect indirectly through activation of immune cells. It has many times been showed that both the antitumor effects and the anti-cancer effects of *Inonotus obliquus* happen mainly because of the enchased phagocytosis and nitric oxide production in macrophages, resulting in immune-stimulating activity. The immune-stimulating activities of endo-polysaccharide in *Inonotus obliquus* are similar to those of lipopolysaccharide (LPS), a typical immune activation compound in gram-negative bacteria. Inonotus obliquus has also shown relatively strong inhibition of mutagenic activity by pure compounds isolated from the ethyl acetate fraction of the fungus. This suggests that these subfractions can protect the DNA or RNA in cells from mutagens.

Polysaccharides, polyphenols, triterpenoids and steroids in *Inonotus obliquus* are having antioxidant activity, which means that Inonotus obliquus can protect the cells against oxidative stress.

Factors that affect the immune-stimulating activity are the chemical composition, configurations, linkage type, and physical properties of polysaccharide. Even the molecular mass of compounds is closely related to the biological activities of *Inonotus obliquus.*

# REFERENCES

## CHAGA MUSHROOM

**1. Wasser S.** *Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides. Appl Microbiol Biotechnol. 2003;60(3):258-74.*

**2. Ham S, Kim S, Moon S, Chung MJ, Cui C, Han E, et al.** *Antimutagenic effects of subfractions of chaga mushroom (inonotus obliquus) extract. Mutation Research/Genetic Toxicology and Environmental Mutagenesis. 2009 1/10;672(1):55-9.*

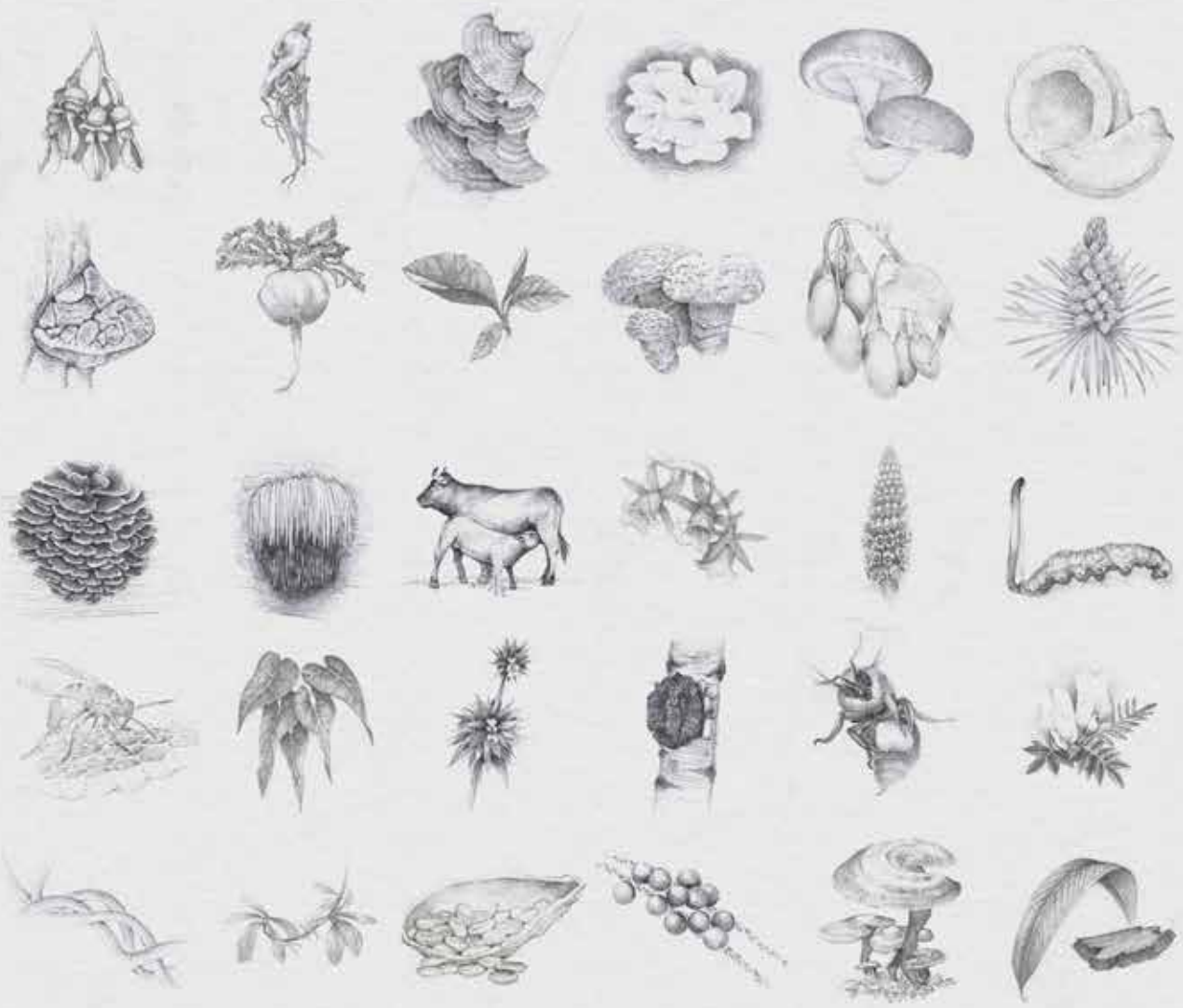
**3. Kim YO, Han SB, Lee HW, Ahn HJ, Yoon YD, Jung JK, et al.** *Immuno-stimulating effect of the endo-polysaccharide produced by submerged culture of inonotus obliquus. Life Sci. 2005 9/23;77(19):2438-56.*

**4. Fan L, Ding S, Ai L, Deng K.** *Antitumor and immunomodulatory activity of water-soluble polysaccharide from inonotus obliquus. Carbohydr Polym. 2012 10/1;90(2):870-4.*

**5. Kim YO, Park HW, Kim JH, Lee JY, Moon SH, Shin CS.** *Anti-cancer effect and structural characterization of endo-polysaccharide from cultivated mycelia of inonotus obliquus. Life Sci. 2006 5/30;79(1):72-80.*

**6. Cui Y, Kim D, Park K.** *Antioxidant effect of inonotus obliquus. J Ethnopharmacol. 2005 1/4;96(1–2):79-85.*

**7. Lee I, Kim Y, Jang Y, Jung J, Yun B.** *New antioxidant polyphenols from the medicinal mushroom inonotus obliquus. Bioorg Med Chem Lett. 2007 12/15;17(24):6678-81.*



## FIND MORE FSF SCIENCE ARTICLES ONLINE

WWW.FOURSIGMAFOODS.COM/SCIENCE