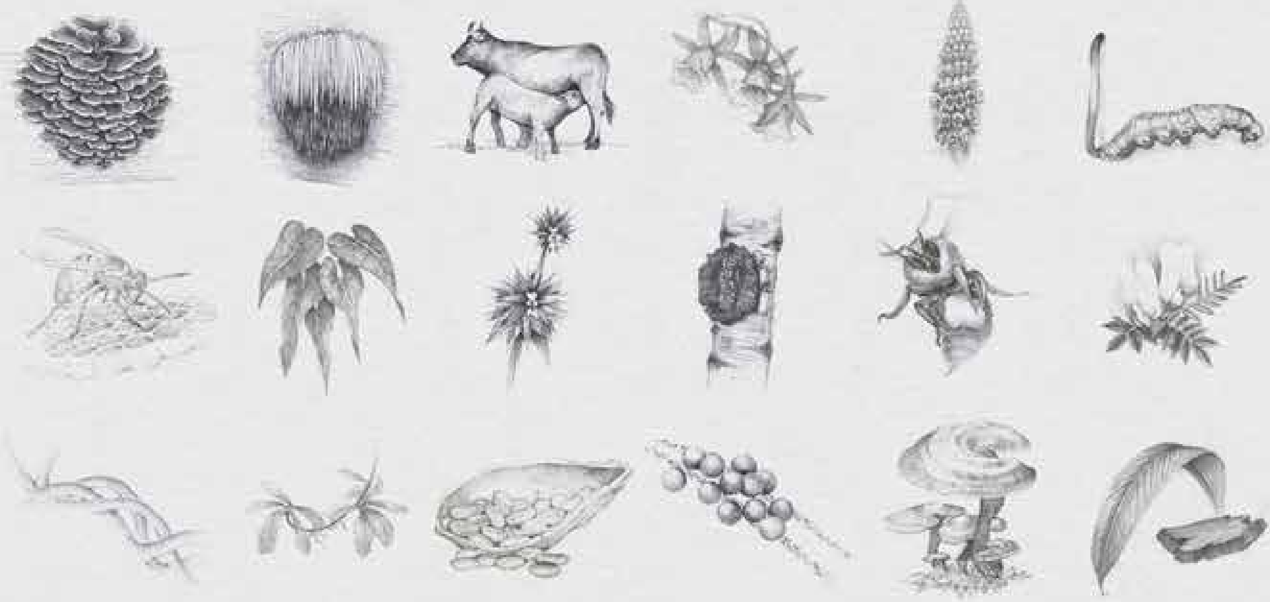
SCIENCE

CHAGA MUSHROOM



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INONOTUS OBLIQUUS

Basiodiomycota fungus comprise a vast and yet largely untapped source of powerful new pharmaceutical products. In particular, and most importantly for modern medicine, these fungi represent an unlimited source of polysaccharides with anti-tumor and immune-stimulating properties. Many, if not all, Basidiomycota contain biologically active polysaccharides in fruiting bodies and cultured mycelium[1].

The white-rot fungus known as chaga (*Inonotus obliquus*) belongs to the Hymenochaetaceae family of Basidiomycota[2]. This fungus is usually observed in nature as a sterile conk (i.e., sclerotium) on Betula tree species such as birch[3]. Chaga mushrooms have been used in folk medicine to treat cancer in Russia, western Siberia, Asia, and North America. It has been shown that Chaga mushrooms contain many steroids and phenolic compounds and that the mushroom also has various biological activities that suggest anti-bacterial, hepato-protective, and cancer-fighting properties. Several compounds used in cancer treatment have been found in natural products of chaga[2, 3]. This brief summary will highlight recent scientific study of this medically important fungus .

ANTI-MUTAGENIC AND ANTI-CANCER PROPERTIES

Many different chemotherapeutic options are now available to cancer sufferers. However, the great majority of chemical compounds that are cytotoxic to cancer cells are also toxic to normal cells. Thus, there is considerable interest in developing cancer therapies that reduce or eliminate the toxic side effects of treatment. Recent research has shown that polysaccharides extracted from plants, fungi, algae, and animals have low toxicity and show a wide range of beneficial effects such as tumor inhibition, scavenging of free radicals, and immunomodulation (4). Moreover, mushroom polysaccharides do not attack cancer cells directly but instead activate immune responses that inhibit tumor growth. The antitumor action of polysaccharides requires an intact T-cell component; their activity is mediated through a thymus-dependent immune mechanism[1].

The endo-polysaccharide from *I. obliquus* sclerotia exhibits anti-cancer effects directly through inhibition of cancer cells, whereas polysaccharide from mycelia produces an effect indirectly through activation of immune cells. Sclerotia extracts of *I. 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].

Kim et al.[5] conducted a trial that tested the effects of *I. obliquus* polysaccharide on mice with implanted melanoma. Specifically, mice were treated with either an intraperitoneal endo-polysaccharide that is a special activator of B cells and macrophages, or they were fed orally with the same substance but in different quantities. Life threatening toxicity was not detected in either group of mice treated with the endo-polysaccharide. Indeed, mice that survived for at least 60 d did not show any tumor growth, with the intraperitoneal group showing a stronger treatment effect than the orally fed group. Further research suggests that endo-polysaccharide activate peritoneal macrophages and induce the production of host defense molecules such as nitric oxide and cytokines. Nitrite has been identified as a key molecular factor in the destruction of tumor cells by activated macrophages. The nitrite production of purified endo-polysaccharide markedly increases compared to crude endo-polysaccharide, indicating that purified endo-polysaccharides enhance phagocytosis and foster production of nitric oxide in macrophages, resulting in immune-stimulating activity. Thus, the cancer-fighting properties of endo-polysaccharide in tumor-bearing mice is probably related to stimulation of the immune system[5].

Other workers observed that *I. obliquus* extract has antitumorigenic and antimutagenic properties. Ham et al.[2] showed that purified compounds (subfractions) isolated from the ethyl acetate fraction of *I. obliquus* relatively strongly inhibit mutagenesis in a dose-dependent manner. This finding suggests that *I. obliquus* subfractions may protect DNA or RNA from cancer-causing mutagens. Alternatively, subfractions may inactivate mutagenic precursors by hindering their transformation into carcinogens. Thus, subfractions of *I. obliquus* extracts show strong anti-mutagenic effects and may therefore be useful as an ingredient in anti-cancer foods[2].

IMMUNE-STIMULATING EFFECTS

Lymphocytes T and B are two important classes of immunologically active cells. The former is mainly responsible for cellular immunity while the latter is the only cell capable of producing antibodies. Macrophage is the most important phagocyte and it plays an essential role in host defense against any type of invading cells including tumor cells. TNF-α plays an important role in tumoricide 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 polysaccharides isolated from plants or fungi are immune-activators. The immune system becomes activated by the polysaccharides via stimulation of cell types T, and B, natural killer (NK) cells, and macrophages[1, 4].

Fan et al.[4] found that in vivo water-soluble polysaccharide from *I. obliquus* significantly enhances the immune response of tumor-bearing mice. In particular, in vivo water-soluble polysaccharide enhanced lymphocyte proliferation and improved the ability of ConA-induced lymphocyte, which activates the T cells, to proliferate. At the same time, it was found that water-soluble polysaccharide could mediate phagocytosis and increase the production of TNF-α compared with control. These results suggest that water-soluble polysaccharide of *I. obliquus* may indirectly mediate antitumor activity by improving immunologic function[4].

Lipopolysaccharide (LPS) is an important constituent of the cell membrane of gram-negative bacteria and may act as endotoxins. When LPS invades the human body, it activates the immune system. Some reports have shown that the immune-stimulating activities of endo-polysaccharide in *I. obliquus* were similar to those of LPS. For example, they both activate B cells and macrophages but not T cells. However, there is a difference in the activating mechanisms of *I. obliquus* polysaccharide and LPS. Specifically, 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

In general, compounds that strongly inhibit mutagenicity also efficiently scavenge free radicals[2]. Mushrooms usually contain a wide variety of free radical scavenging molecules such as polysaccharide and polyphenols[6].

Ham et al.[2] determined the antioxidant activity of the *I. obliquus* subfractions using DPPH, a stable free radical that is widely used as a substrate to evaluate the antioxidant activity of biological compounds. In this study it was demonstrated that different extracts and fractions of *I. obliquus* and its insoluble polysaccharides have DPPH radical-scavenging activity. In a similar study, Lee et al.[7] described from *I. obliquus* subfractions six major polyphenol antioxidants, three new inonoblins, and three known phelligridings. The antioxidant activities of the polyphenols were evaluated with three different assays including the DPPH radical scavenging activity assay, the ABTS radical scavenging activity assay and the superoxide radical anion scavenging activity assay. The polyphenols exhibited significant scavenging activity

against the ABTS radical cation and DPPH radical, and showed moderate activity against the superoxide radical anion. Lee and coworkers also compared the compounds from *I. obliquus* with synthetic antioxidants such as butylated hydroxyanisole (BHA), trolox (a water-soluble derivative of vitamin E), and caffeic acid, each of which are well-known antioxidants that can serve as experimental controls. Each isolated polyphenol exhibited higher ABTS cation radical scavenging activity than trolox and caffeic acid, and some of the compounds showed stronger effects than synthetic BHA. Most of the *I. obliquus* compounds were, however, less active than caffeic acid, trolux, and BHA in the test of DPPH radical scavenging effect.

Moreover, *I. obliquus* polysaccharides and polyphenolic extracts are similar to triterpenoids and steroids which have antioxidant activity. Of these substances, the polyphenolic extract has the strongest antioxidant activity, with free radicals scavenged by single-electron transfer and with a strong protective activity against a powerful oxidizer like hydrogen peroxide. This means that polyphenols of *I. obliquus* can protect cells against oxidative stress.

Cui et al.[(6)] conducted a study with *I. obliquus* extract that contained triterpenoids, the steroids lanosterol and inotodiol, trametenolic acid, and ergosterol peroxide. Each of these compounds exhibited a relatively strong antioxidant effect. These results suggest that triterpenoids and steroids may account for the free radical scavenging effect of *I. obliquus*. The superoxide radical scavenging activity of the *I. obliquus* extracts was also generally quite high. However, the triterpenoids and steroids extract did not show any protective effect


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against hydrogen peroxide-induced oxidative stress, although they did show 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 antitumor activity differ greatly in chemical composition, configuration, 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 type of polysaccharide linkage is another important factor in antitumor and immune-stimulating activities. For instance, a triple-helical tertiary conformation of medicinal mushroom $\beta(1\rightarrow3)$-glucans is known to have significant immune-stimulating effects[1].

Molecular mass of mushroom polysaccharides is also thought to strongly influence biological activities. 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 *I. obliquus*. According to a study by Kim et al.[3], the most water-soluble endo-polysaccharides smaller than 50 kDa had low activities. The 1100 kDa endo-polysaccharide seemed to be responsible for the high activities of polysaccharides from *I. obliquus* mycelia.

It has also been suggested that polysaccharide activity may be closely related to cell age[3].

CONCLUSIONS

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The white-rot fungus *I. obliquus*, also known as chaga, is usually found in the wild as a sterile conk (sclerotia) on birch trees. *Inonotus obliquus* has long been a folk remedy in areas like Russia and western Siberia where it is used to treat many different ailments including cancer. Submerged mycelial culture is the preferred method for producing more *I. obliquus* polysaccharide; however, there are differences between the sclerotial and the mycelial fungus. For instance, sclerotial endo-polysaccharide exhibits anti-cancer effects directly through inhibition of cancer cells, whereas mycelial products work indirectly through activation of immune cells. The antitumor and anticancer effects of *I. obliquus* are realized as enhanced phagocytosis and increased nitric oxide production in macrophages, which in turn induces immune-stimulating activity. The immune-stimulating activities of *I. obliquus* endo-polysaccharide are similar to those of lipopolysaccharide (LPS), a typical immune activation compound found in gram-negative bacteria. Purified *I. obliquus* compounds isolated from the ethyl acetate fraction of the fungus also show relatively strong inhibition of mutagenesis. This finding suggests that these subfractions can protect the DNA or RNA in cells from mutagens.

Polysaccharides, polyphenols, triterpenoids, and steroids in *I. obliquus* show antioxidant activity. Thus this fungus can be used to protect cells against oxidative stress. Factors that affect immune-stimulating activity are chemical composition, configuration, linkage type, and physical properties of polysaccharide. And finally, molecular mass of compounds is closely related to the biological activities of *I. obliquus*.

DISCLAIMER

Statements throughout this publication have not been evaluated by the FDA. These products are not intended to diagnose, treat, cure or prevent any disease process.

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