Shiitake (*Lentinus edodes*)

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INTRODUCTION

Shiitake mushroom, the common Japanese name for *Lentinus edodes* (Fig. 1), derives from the mushroom associated with the shii tree (*Castanopsis cuspidate* Schottky) and *take*, the Japanese word for mushroom (Table 1). Because Japan is the world leader in production of this type of mushroom, the mushroom is now widely known by this name. These mushrooms are renowned in Far East countries (e.g., Japan, China, Korea) as a food and medicine for thousands of years. In the year 199 A.D., Kyusuyu, a native tribe of Japan, offered the Japanese Emperor Chuai a shiitake mushroom. Even older documents record its use in ancient China, where it was referred to as ''ko-ko'' or ''hoang-mo.''[1] The cultivation of this mushroom has been practiced for a thousand years, with its cultivation originating in China during the Sung Dynasty (960–1127). Both history and legend credit Wu San Kwung as the originator of shiitake cultivation. Almost every mushroom-growing village in China has a temple in his honor.[2] In 1313, Chinese author Wang Cheng recorded shiitake-growing techniques in his *Book of Agriculture*. He described how to select a suitable site, choose appropriate tools, and cut down the trees on which one could cultivate the mushrooms. He outlined the basic methods as follows: Cut the bark with a hatchet and cover the logs with soil. After 1yr, top the soil and water frequently. Beat the logs with a wooden club to induce mushroom production. The mushrooms will appear after a rain.[2,3]

Shiitake mushroom cultivation techniques were probably introduced to Japanese farmers by the Chinese between 1500 and 1600 A.D.[4]

At present, shiitake is one of the five most cultivated edible mushrooms in the world.[5] Its production (2 million tons) is second only to button mushroom *Agaricus bisporus*. Grown mainly in East Asia, shiitake is now arousing interest worldwide.[5–8] Increasing markets have been spawned, partly by the exotic and well-appreciated taste of shiitake, and partly by advances in research that has demonstrated its significant medicinal properties. Shiitake mushroom is becoming popular in nutritional and medicinal products throughout Asia, Europe, and North America.

HABITAT AND DISTRIBUTION

Gregarious on fallen wood of a wide variety of deciduous trees, especially shii, oak, chestnut, beech, maple, sweet gum, poplar (aspen, cottonwood), alder, hornbeam, ironwood, chinquapin, mulberry (*Castanopsis cuspidate*, *Quercus, Castanea*, *Fagus, Acer, Liquidamber, Populus, Diospyros, Alnus, Carpinus, Morus*) in a warm, moist climate. Most of these are raised for artificial cultivation of shiitake mushroom.

L. edodes occurs naturally throughout Southeast Asia. It has been reported from China, Japan, Korea, Vietnam, Thailand, Burma, North Borneo, the Philippines, Taiwan, and Papua New Guinea.[7,8]

EDIBILITY AND NUTRITIONAL VALUE

Shiitake are traditionally well-known edible mushrooms of high nutritious value. Raw or dried forms, used in Chinese curative powers of shiitake mushroom, are legendary. It was stated in *Ri Youg Ben Cao*, Vol. 3 (1620), written by Wu-Rui of the Ming Dynasty, ''shiitake accelerates vital energy, wards off hunger, cures colds, and defeats body fluid energy.'' In later years, it was found that the mushroom contained various important nutrients. Moreover, recent scientific investigations have isolated many compounds and have found evidence of their health promotion activities.[1,7–10]

Shiitake mushrooms have excellent nutritional value. Their raw fruit bodies include 88–92% water, protein, lipids, carbohydrates as well as vitamins and minerals. It should be noted that amounts of nutrients and biologically active compounds differ in various strains and are affected by substrate, fruiting conditions, and methods of cultivation. On a dry weight basis, they have a relatively high nutritional value when compared to commonly consumed vegetables.

Dried shiitake mushrooms are rich in carbohydrates and protein. They contain 58–60% carbohydrates, 20–23% protein (digestibility of 80–87%), 9–10% fiber, 3–4% lipids, and 4–5% ash. The mushroom is a good source of vitamins, especially provitamin D_2

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Encyclopedia of Dietary Supplements DOI: 10.1081/E-EDS-120024880
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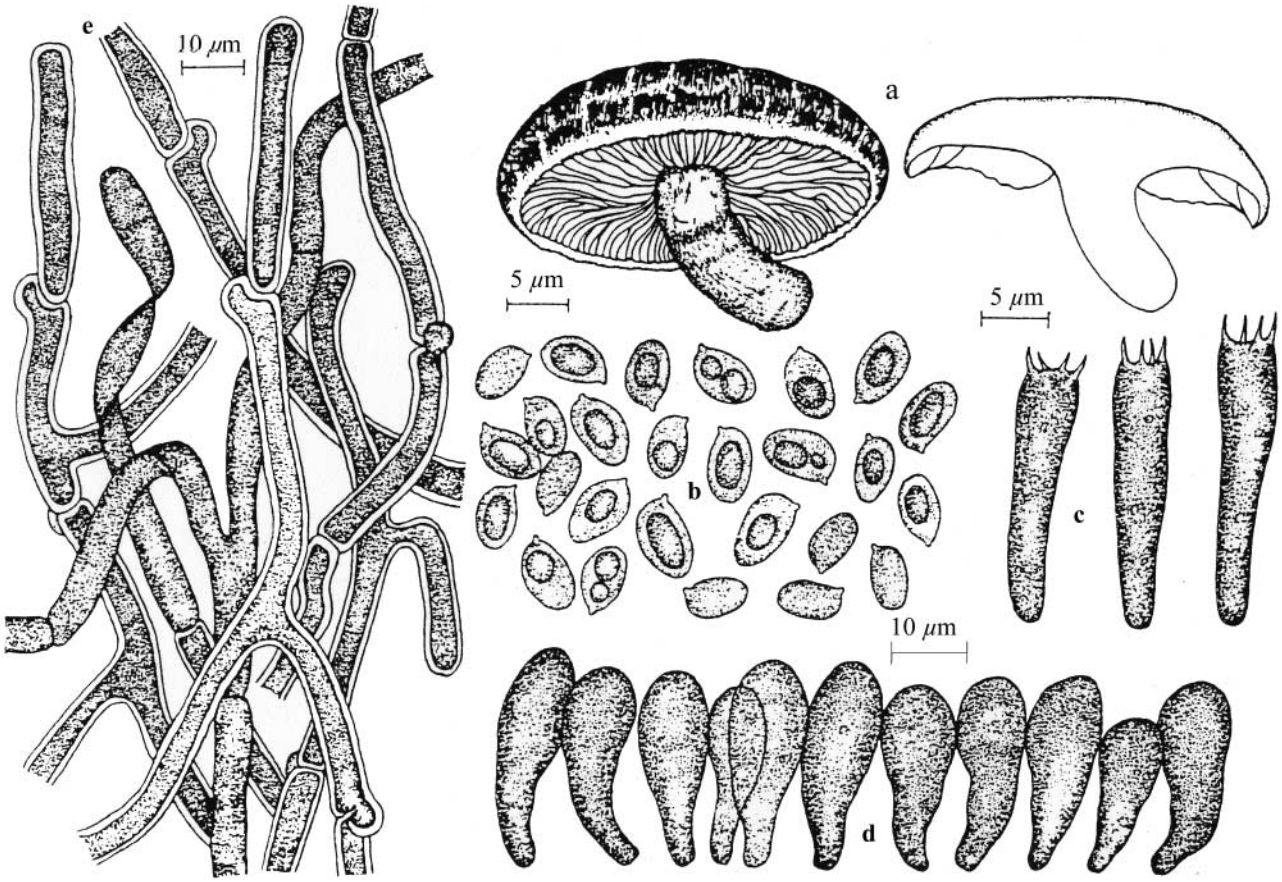

Fig. 1 Shiitake mushroom [*L. edodes* (Berk.) Singer]. a—fruit body, b—spores, c—basidia, d—cheilocystidia, e—elements of pileal cuticle.

(ergosterol), 325 mg%, which under ultraviolet (UV) light and heat yields calciferol (vitamin D_2). It also contains B vitamins, including B_1 (thiamine), B_2 (riboflavin), B_{12} (niacin), and pantothenic acid.[1,3,9,10] Minerals found include Fe, Mn, K, Ca, Mg, Cd, Cu, P, and Zn. Analysis of dried cultured shiitake mycelium gives the following mineral concentrations (in mg/g of dry weight): K, 15.1; Ca, 22; Mg, 44–78; Mn, 1.2; Cd, 0.96; Fe, 2.36; Ni, 52.5; Cu, 89.1; P, 281; Zn, 282; Ge, 3; Br, 11.4; and Sr, 164.

Water-soluble polysaccharides amount to 1–5% of the dry weight of the shiitake mushroom. In addition to glycogen-like polysaccharides, (1-4)-,(1-6)-α-D-glucans and antitumor polysaccharides, lentinan, (1-3)-, (1-6)-β-bonded heteroglucans, heterogalactans, heteromannans, xyloglucans, etc., have been identified. The mushrooms' indigestible polysaccharides, which serve as dietary fiber, include heteroglycan, polyuronide, β-glucan as well as chitin. Among the free sugars present are trehalose, glycerol, mannitol, arabitol, mannose, and arabinose.[1,7–10]

In shiitake mushrooms, dietary fiber consists of water-soluble materials such as β-glucan and protein and water-insoluble substances extractable only with salts, acids, and alkalies such as polyuronide (acidic polysaccharide), hemicellulose, β-glucan with heterosaccharide chains, lignin, and chitin present as cell wall constituents.

The fatty acids account for 3.38% of the total lipids.[9,16] Their composition is as follows: linoleic acid (18 : 2), 72.8%; palmitic acid (16 : 0), 14.7%; oleic acid (18 : 1), 3.0%; tetradecenoic acid (14 : 1), 1.6%; stearic acid (18 : 0), 0.9%; and myristic acid (14 : 0), 0.1%.

The aroma components include alcohols, ketones, sulfides, alkanes, fatty acids, etc. The major volatile flavor contributors are *matsutakeol* (octen-1-ol-3) and ethyl-*n*-amyl ketone. The characteristic aroma of shiitake mushrooms was identified as 1,2,3,5,6-pentathiepane. According to Mizuno,[9] the components responsible for the delicious flavor are monosodium glutamate, 5′-nucleotides, free amino acids, lower molecular weight peptides, organic acids, and sugars. Their relative ratios are responsible for the variation in flavor naturally seen in this mushroom. Organic acids contributing to the flavor of shiitake mushroom include malic acid, fumaric acid, α-keto-glutaric acid, oxalic acid, lactic acid, acetic acid, formic acid, and glycolic acid.

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Table 1 Mycological data for shiitake

Basidiomycotina	
Homobasidiomycetes	
Agaricomycetiae	
Agaricales	
Family: Pleurotaceae	
Genus: *Lentinus*	
Lentinus edodes (Berk.) Singer, Mycologia, 1941 33, 451 (Fig. 1)	
Basyonym	*Agaricus edodes* Berk., J. Linn. Soc. Bot. 1877, 16, 50.
Synonyms	*Collybia shiitake* Schroet., Garenfl. 1886, 35, 105.
	Armillaria edodes (Berk.) Sacc., Syll. Fung. 1887, 5, 79.
	Agaricus russaticeps (Berk.) apud Cooke, Grevillea 1889, 16, 106.
	Lepiota shiitake (Schroet.) Tanaka, Japan Bot. Mag. 1889, 3, 159.
	Lentinus tonkinensis Pat., J. Bot. Paris 1890, 4, 14.
	Mastaleucomyces edodes (Berk.) O. Kuntze, Rev. Gen. Pl. 1891, 2, 861.
	Pleurotus russaticeps (Berk.), Sacc., Syll. Fung. 1891, 9, 48.
	Cortinellus shiitake (Schroet.) P. Henn., Not. Knigl. Bot. Gard. Mus. Berl. 1899, 2, 385.
	Tricholoma shiitake (Schroet.) Singer, Ann. Mycol. 1936, 34, 332.
	Cortinellus edodes (Berk.) S. Ito et Imai, Journ. Fax. Agr. Hokkaido Imp. Univ. 1938, 43, 55.
	Lentinula edodes (Berk.) Pegler, Kavaka 1975, 3, 20.
English names	Black forest mushroom, black oak mushroom, golden oak mushroom, snake butter, pasania mushroom, oakwood mushroom, Japanese forest mushroom
Japanese name	Shiitake
Chinese names	Shiang-gu, Shing ku, Hua Gu, Xiangu, Hoang-mo

A detailed description of the shiitake mushroom can be found in Refs.[1,4,7].

RELATED SPECIES

Artificial Cultivation

The shiitake mushroom is commonly considered as a species of the genus *Lentinus* Fr. As noted in the below synonymy, *L. edodes* has at various times been assigned to 10 genera (*Agaricus*, *Collybia*, *Armillaria*, *Lepiota*, *Lentinus*, *Mastaleucomyces*, *Pleurotus*, *Cortinellus*, *Tricholoma*, and *Lentinula*). While specialists in medicinal mushrooms and cultivation are familiar with shiitake mushrooms as *L. edodes*, some alternative taxonomic classifications are discussed in Refs.[11–15] Although the mushroom had been grown in Asian countries for centuries, the interest there, as well as in the Western countries, has increased rapidly since World War II, especially in the last 15–20 yr. Its cultivation is now a worldwide multimillion dollar industry.

The process for producing shiitake mushroom fruiting bodies (Fig. 2) is the same as for other cultivated edible mushrooms and can be divided into two major stages. The first stage involves the preparation of the fruiting culture, stock culture, mother spawn, and planting spawn, and the second stage entails the preparation of the growth substrates for cultivation. Currently, the methods most widely adopted for commercial production are wood log and synthetic sawdust bag.[3,6–8,16] A discussion of the cultivation methods used is beyond the scope of this review. Interested readers may refer to the references cited above; growth parameters for cold- and warm-weather strains are given in Ref.[8]

PRECLINICAL STUDIES

Therapeutic Applications

This section mainly discusses preclinical in vitro and in vivo (animal) studies.

Shiitake is one of the best-known and best-characterized mushrooms used in medicine. It is the source of several well-studied preparations with proven pharmacological properties, especially the polysaccharide

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Fig. 2 Shiitake mushroom [*L. edodes* (Berk.) Singer]: cultivated fruiting bodies.

lentinan, shiitake mushroom mycelium, and culture media extracts (LEM, LAP and KS-2).[7,9,16–19]

Anticarcinogenic and Antitumor Effects

Using methods of fractionation and purification of polysaccharides, Chihara et al.[20–22] isolated a water-soluble antitumor polysaccharide from fruiting bodies of shiitake, which was named ''lentinan'' after the genus *Lentinus* to which the shiitake mushroom belongs. Chihara was one of the first to report on the antitumor properties of the mushroom, stating that lentinan ''was found to almost completely regress the solid type tumors of Sarcoma 180 and several kinds of tumors including methylchloranthrene-induced fibrosarcoma in synergic host–tumor system A.''[21,22] The antitumor effect of lentinan was originally confirmed by using Sarcoma 180 transplanted in CD-1/ICD mice.[20] Later, it showed prominent antitumor activity not only against allogenic tumors such as Sarcoma 180, but also against various synergic and autochthonous tumors, and it prevented chemical and viral oncogenesis.[23] The molecular formula of β-D-glucan lentinan is $(C_6H_{10}O_5)_n$, and the mean molecular weight is about one million (-5×10^5 Da); $[\alpha]_D$ + 20–22° (NaOH). Its structure was confirmed as β-(1-3)-D-glucopyranan with a branched chain of β-(1-6)-monoglycosyl (branching degree: 2.5°), showing a right-handed triple helix.[7,9,17,18] It is water soluble, heat stable, and alkali labile. That is, β-D-glucan binds to lymphocyte surfaces or serum-specific proteins, which activate macrophage, T-helper cells, natural killer (NK) cells, and other effector cells. All these increase the production of antibodies as well as interleukins (IL-1, IL-2) and interferon (IFN-γ) released upon activation of effector cells.[19,24] Thus, the carcinostatic effect of lentinan results from the activation of the host's immune system. In animal testing of carcinostatic activity, intraperitoneal (i.p.) administration is used, but oral administration (p.o.) is occasionally effective.

The purified polysaccharide has been shown in animal studies to produce strong tumor regression and even the disappearance of sarcoma tumors in 5 weeks, ascite hepatoma 134,[18,19,25] and Ehrlich carcinoma as well as a number of other experimentally induced cancers in allogenic, syngeneic, and autologous hosts. It also exhibits preventive activity against chemical carcinogenesis. Injections of lentinan into mice produced either an 80% reduction in tumor size or complete regression in most of the animals tested. Moreover, an intact immune system and a functioning thymus gland were found to be requisite for its anticancer effect.[11,12] When immunosuppressive agents such as β-benzylthioguanosine or X-radiation were given with lentinan, the antitumor effect decreased. The polysaccharide has also been found to restore the enzyme

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activity of X-prolyl-dipeptidyl-aminopeptidase, which can be depressed in cancer patients and in mice with implanted tumors.[26]

Laboratory tests seem to indicate a role for the adrenal–pituitary axis and central peripheral nervous system including serotonin, 5HT, histamine, and catecholamies in lentinan's antitumor activity.[1,10,17,24]

The oral administration of the polysaccharide to AKR mice exerted strong antitumor activity resulting in raised levels of lymphocytokines, such as IFN-γ, tumor necrosis factor (TNF-α) and IL-1 α. Tissue cultures of murine macrophages CRL-2019, B-lymphocytes HB-284, and T-lymphocytes DRL-8179, which were treated with lentinan, showed high levels of activation using flow cytometry. Lentinan-activated immunocytes, particularly the T-helper cells, might render the physiological constitutions of the host highly cancer- and infection resistant. Adoptive immunotherapy of the immunodeficient mice such as the nude (athymic) mice, B-cell deficient mice, and severe combined immunodeficient (SCID) mice via the transfer of the lentinan-activated immunocytes resulted in the inhibition of tumor growth. Lentinan appeared to represent a unique class of host defense potentiators (HDP), protecting the hosts from the side effects of conventional therapeutic measures and improving various kinds of immunological parameters with no toxic side effects in animal models.[19,24,27,28]

LEM and LAP Extracts from Shiitake Mushroom Mycelium and Culture Media

L. edodes mycelium (LEM) is prepared from an extract of the powdered mycelia of the shiitake mushroom. Its yield is about 6–7 g/kg of medium. The precipitate obtained from a water solution of the mycelium by adding four volumes of ethanol was named LAP. The yield of LAP is ≈0.3 g/g of LEM.

L. edodes mycelium and LAP are glycoproteins containing glucose, galactose, xylose, arabinose, mannose, and fructose.[9] The former also contains various nucleic acid derivatives, vitamin B compounds especially B_1 (thiamine), B_2 (riboflavin), and ergosterol.[7,8]

In 1990, an immunoactive substance, EP3, was obtained by fractionation of LEM. EP3 is a lignin complex composed of about 80% lignin, 10% carbohydrates, and 10% protein. After removal of the last two components, biological activity was not affected, but when lignin is removed, activity was reduced. Therefore, the active substance is believed to be a water-soluble lignin containing numerous carboxyl groups.[9,16]

Both LEM and LAP have demonstrated strong antitumor activities orally and by injection to animals and humans. They were shown to act by activating the host's immune system and are also useful for the treatment of hepatitis B.[9,14–16]

KS-2-α-Mannan Peptide

Polysaccharide KS-2 (MW 6–9.5 × 10^4 $[\alpha]_D$ + 62°; C = 0.5, water) was obtained by extraction of cultured mycelia of shiitake mushroom (strain KSLE 007) with hot water, followed by precipitation with ethanol.[9,16,29] The product is an α-mannan peptide containing the amino acids serine, threonine, alanin, and proline (as well as residual amounts of the other amino acids). The polysaccharide was shown[29] to be effective on Sarcoma 180 and Ehrlich's carcinoma, either i.p. or p.o., and to act via interferon-inducing activity. The acute LC_{50} or KS-2 was found to be extremely low in mice, more than 12,500 mg/kg when administered orally.

The mechanism of action of KS-2 is not yet clear, but the results showed no direct KS-2 cytocidal effect against the tumor cells in vitro. Its antitumor activity was observed to be higher at the lower inoculum size of tumor cells, regardless of the routes of KS-2 administration (60% survival rate at 5 × 10^3 tumor cells/mouse, 10% survival at 1 × 10^6 tumor cells/mouse). The results also showed that the antitumor activity of KS-2 in mice was always accompanied by the induction of interferon in the sera. Furthermore, preliminary findings indicated that macrophages obtained from KS-2 treated mice exhibited tumoricidal activity,[10,16,30] and it was reported that macrophage activation became tumoricidal when incubated in vitro with interferon. Considering these findings, the antitumor activity of KS-2 may be explained by macrophage activation with or without interferon induced by KS-2.

Immune-Modulating Effects

As was stated earlier, lentinan and other polysaccharides from shiitake mushrooms do not attack cancer cells directly, but produce their antitumor effects by activating different immune responses in the host. Lentinan, for example, appears to act as an HDP, which is able to restore or augment the responsiveness of host cells to lymphocytokines, hormones, and other biologically active substances by stimulating maturation, differentiation, or proliferation of cells involved in host defense mechanisms.[19,24] Host defense potentiators are functionally different from biological response modifiers. Thus, lentinan is able to increase host resistance against various kinds of cancer and infectious diseases, including acquired immuno deficiency syndrome (AIDS).[7,28]

The initial interactions of lentinan in the human body or animals are not presently known. However,

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there is a transitory but notable increase in several serum protein components in the α- and β-globulin region, namely, complement C3, hemopexin, and ceruloplasmin.[7,10,19,24]

Lentinan stimulates various kinds of NK cell-, T cell-, B cell-, and macrophage-dependent immune reactivities. Its antitumor effect is abolished by neonatal thymectomy and decreased by the administration of antilymphocyte serum, supporting the concept that the polysaccharide requires immunocompetent T-cell compartments. The effect of lentinan was also inhibited by antimacrophage agents, e.g., carrageenan. Unlike other well-known immunostimulants, lentinan is in a unique class of distal tubular (DT)-cell-oriented assistant, in which macrophages play some part.[7,10,19,24]

For example, lentinan can activate NK cells in vitro in the same concentrations that are achieved in the blood plasma of patients treated clinically with lentinan.[10,16,24] Natural killer-cell activity is involved in tumor suppression, and while these cells do not stimulate T-killer cell activity or do so only under certain conditions, they are strong T-helper cell stimulants both in vitro and in vivo.[1,7,10,16,19,24] Using the blood of healthy donors and cancer patients, some authors have shown that the polysaccharide is able to stimulate peripheral blood lymphocytes in vitro to increase IL-2-mediated lymphokine-activated killer cell (LAK-cell) and NK cell activity at levels achievable in vivo by administration of clinical doses of lentinan. It has been shown to inhibit suppressor T cell activity in vivo and to increase the ratio of activated T cells and cytotoxic T-cells in the spleen when administered to gastric cancer patients undergoing chemotherapy.[7,10,24]

Many interesting biological activities of lentinan have been reported including: a) an increase in the activation of nonspecific inflammatory responses such as acute phase protein (APP) production, b) vascular dilation and hemorrhage in vivo, c) activation and generation of helper and cytotoxic T-cells, d) augmentation of immune mediators like IL-1 and IL-3, colony stimulating factor(s), and migration inhibitory factor, and e) increasing the capacity of peripheral blood mononuclear (PBM) cells of patients with gastric cancer and producing IL-1α, IL-1β, and a TNF-α.[7,10,19,24,27]

In an in vivo study of rats with peritonitis, combined lentinan–gentamicin treatment had a significantly better survival rate than the controls. Lentinan activated the peritoneal macrophages' secretory activity of active oxygen and produced cytokines, thus enhancing the ability of polymorphonuclear leukocytes (PMNs) to produce active oxygen, which has a bactericidal effect.[31] It also increases peritoneal macrophage cytotoxicity against metastic tumor cells in mice, but not against a highly metastic tumor type.[32] Some patients treated with lentinan for carcinomatous pleuritis or carcinomatous peritonitis have improved with the disappearance of malignancy, while in another group their condition deteriorated or diminished.[33] The polysaccharide can activate the normal and alternative pathways of the complement system and can split C3 into C3a and C3b enhancing macrophage activation.[34]

Many biological reactions are accelerated and induced by lentinan, including the very important phenomenon of infiltration of eosinophils, neutrophils, and granulocytes around target tissues. Fig. 3 shows early responses initiated by it and possible pathways for inflammatory reactions.

Lentinan's immune-activating ability may be linked with its modulation of hormonal factors, which are known to play a role in tumor growth. Aoki[34] showed that the antitumor activity of lentinan is strongly reduced by administration of thyroxin or hydrocortisone. It can also restore tumor-specific antigen-directed delayed-type hypersensitivity reaction (DTHR).

Lentinan is not formally included among the nonspecific immunostimulants (RES stimulants), but it augments the induction of antigen-specific cytotoxic T-lymphocytes, macrophages, and other nonspecific immune responses.

Possible immune system regulating actions of lentinan were summarized by Chihara et al.[23] and are seen in Fig. 4.

Cardiovascular Effects

The major cause of death in Western countries is coronary artery disease, a primary risk factor for which hypercholesterolemia is a factor contributing to hardening of the arteries. In humans, 50% or more of the total cholesterol is derived from de novo synthesis.[18,35,36]

It is known that shiitake mushroom is able to lower blood serum cholesterol (BSC) via a factor known as eritadenine (also called "lentinacin" or "lentysine").

Apparently, eritadenine reduces BSC in mice, not by the inhibition of cholesterol biosynthesis, but by the acceleration of the excretion of ingested cholesterol and its metabolic decomposition. It has been shown to lower blood levels of cholesterol and lipids in animals. When added to the diet of rats, eritadenine (0.005%) caused a 25% decrease in total cholesterol in as little as one week. The cholesterol-lowering activity of this substance is more pronounced in rats fed a high-fat diet than in those on a low-fat diet. Although feeding studies with humans have indicated a similar effect, further research is needed. Hobbs[1,10] and Yang et al.[36] have shown that shiitake mushrooms lowered BSC levels. Various studies have confirmed[1,7,10,16] that the mushroom can lower blood pressure and free cholesterol in plasma, as well as accelerate the accumulation of lipids in the liver by removing them from circulation.

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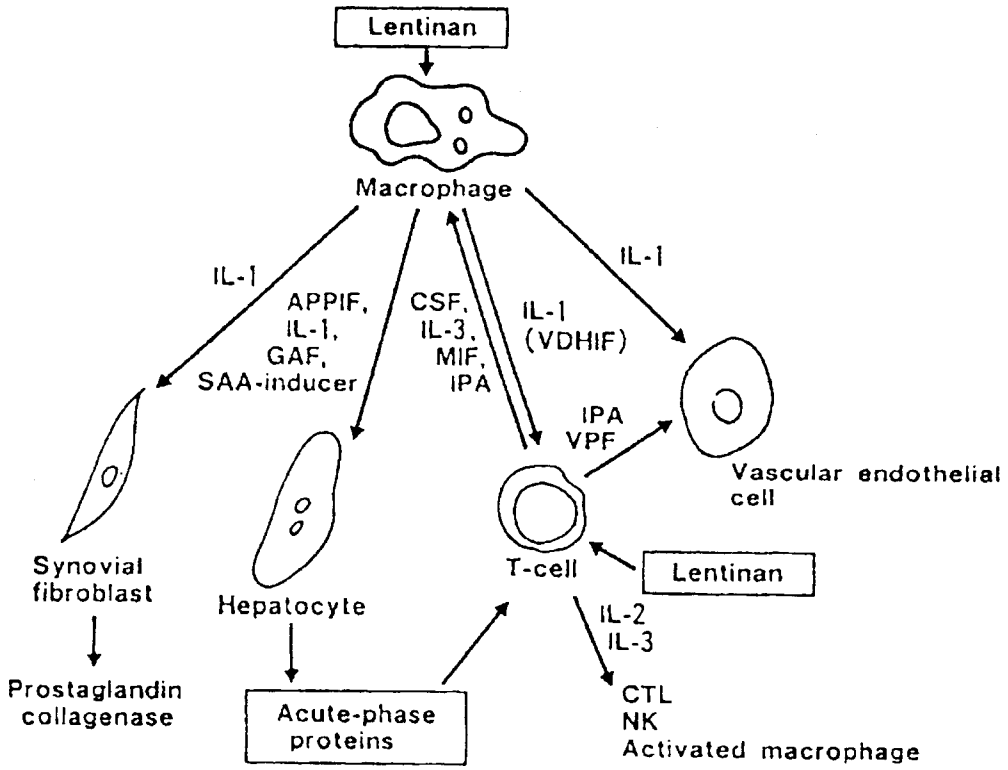

Fig. 3 Early responses initiated by lentinan and a possible pathway for inflammatory reactions. APPIF, acute phase protein-inducing factor; VDHIF, vascular dilatation and hemorrhage-inducing factor; CSF, colony stimulating factor; MIF, migration inhibitory factor; GAF, glucocorticoid antagonizing factor; SAA, serum amyloid A; IPA, plasminogen activator inducer; VPF, vascular permeability factor; CTL, cytotoxic T-lymphocytes; NK, natural killer cells.[16]

Hepatoprotective Effects

The injection of LEM slowed the growth of cancerous liver tumor in rats.[16,18,37] A polysaccharide fraction from shiitake mushrooms demonstrated liver protection in animals as well as the ability to improve liver function and enhance the production of antibodies to hepatitis B.[7,35]

Lentinan improved serum glutamic pyruvic transaminase (SGPT) and completely restored GPT levels in the livers of mice with toxic hepatitis. Crude extracts of shiitake mushroom cultures have demonstrated liver-protecting actions.[10,16,18,35]

Antiviral, Antibacterial, and Antiparasitic Effects

Lentinan and its derivatives are effective against various kinds of bacterial, viral (including AIDS), and parasitic infections.[7,10,18,28] An important area of this polysaccharide research deals with its ability to mobilize the body's humoral immunity to ward off bacterial infections resistant to antibiotics.[7] Many cancer and AIDS patients die of opportunistic infections due to immunodysfunction.[7,27] It is extremely important to protect AIDS patients from these various infections. According to Ref.[39], in vitro studies show that lentinan, when used in combination with azidothymidine (AZT), suppressed the surface expression of human immunodeficiency virus (HIV) on T cells more so than did AZT alone. Lentinan and the sulfated form exhibited potent in vitro anti-HIV activity resulting in inhibition of viral replication and cell fusion. AIDS therapy must include a strategy to enhance the immune system. Among the various therapeutic approaches used, prevention of the development of AIDS symptoms in carriers should be stressed. Based on these in vitro studies, it is possible that such prevention may be realized by the use of HDPs such as lentinan or its related substances. For example, LEM is also useful in the treatment of AIDS. It has been shown to inhibit HIV infections of cultured human T cells, and it potentiates the effects of AZT against viral replication in vitro. The mechanism of its action is not known for certain, but the extract was found to activate macrophages and stimulate the production of IL-1.[7,10,36,39]

Water-soluble lignins from EP3 and EPS4 from shiitake mushroom mycelium have shown antiviral and immunomodulating effects.[40] A water-soluble extract

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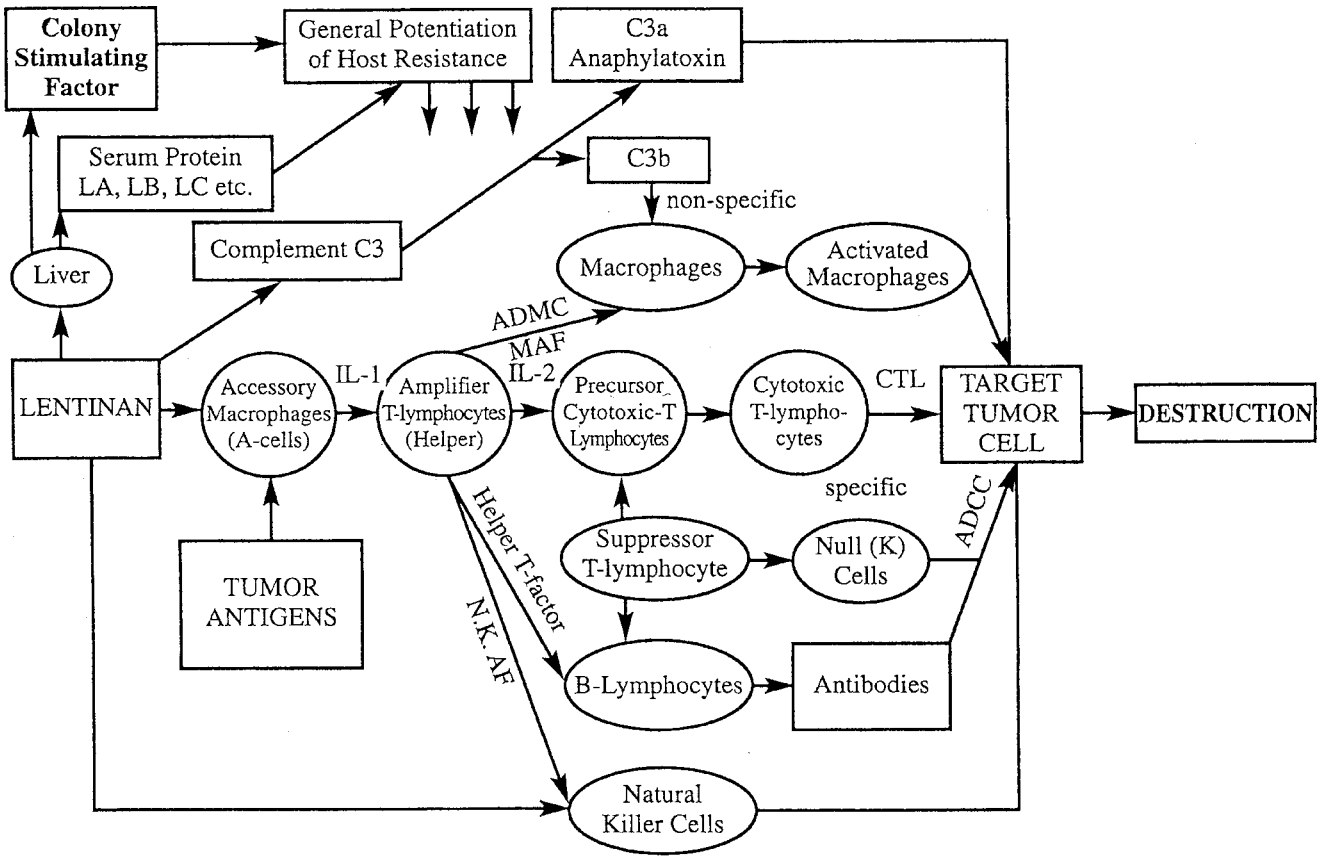

Fig. 4 Possible mode of action of lentinan as HDP. (From Ref.[23].)

of mycelium known as JLS and JLS-18 has the ability to block the release of *herpes simplex* virus type 1 in animals JLS-18-consisting of 65–75% lignin, 15–30% polysaccharides, and 10–20% protein has inhibited the herpes virus both in vitro and in vivo.[41]

In addition, lentinan has shown: a) antiviral activity in mice against vesicular stomatitis virus (VSV) encephalitis virus, Abelson virus, and adenovirus type 12; b) stimulated nonspecific resistance against respiratory viral infections in mice; c) conferred complete protection against an LD75 challenge dose of virulent mouse influenza A/SW15; d) enhanced bronchoalveolar macrophage activity; e) increased resistance against the parasites *Schistosoma japonicum* and *S. mansoni*; f) exhibited activity against *Mycobacterium tuberculosis* bacilli resistant to antituberculosis drugs, *Bacillus subtilis, Staphylococcus aureus, Micrococcus luteus, Candida albicans*, and *Saccharomyces cerevisiae*; and h) increased host resistance to infections with the potentially lethal *Listeria monocytogenes*. Antibacterial polyacetylene compounds, centinamycin A and B, have also been identified in shiitake mushroom. Eritadenine, a compound that affects cholesterol metabolism, also processes antiviral properties.[7,10,35]

It should be noted that a protein fraction of shiitake mushroom fruiting bodies, labeled fruiting body protein (FBP), prevented the infection of plants with tobacco mosaic virus (TMV). The binding of the virus to the plant cells was inhibited by FBP.[7,9,16]

HUMAN CLINICAL STUDIES AND MEDICINAL USES

In the last 15–20 yr, shiitake mushroom has been subject to various clinical studies in humans and is thought to be beneficial for a wide variety of disorders including different types of cancer, heart disease, hyperlipidemia (including high blood cholesterol), hypertension, infectious disease, and hepatitis. The mushroom is used medicinally for diseases involving depressed immune function (including AIDS), cancer, environmental allergies, fungal (especially *Candida*) infection, frequent flu and colds, bronchial inflammation, and regulating urinary incontinence.

It was shown that the success of immune adjuvant in therapy depends on the type of cancer (location) being treated, the individual's general health,

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immunological and hormonal status as well as the individual's constitution.

Lentinan was demonstrated to have antitumor activity as well as to increase the survival time of patients with inoperable gastric cancer[10] and women with recurrent breast cancer following surgical therapy (for details on protocols, see Refs.[7,10,18]). According to Refs.[42,43], when the polysaccharide is administered once or twice a week with chemotherapy to a patient with progressive cancer but with no serious liver, kidney, or bone marrow dysfunction, it produced a statistically significant improvement in immune and anticancer activity when compared to chemotherapy alone. Two hundred seventy-five patients with advanced or recurrent gastric cancer were given one of two kinds of chemotherapy (mitomycin C with 5-fluorouracil or tegafur) either alone or with lentinan injections. Statistically, the best results were obtained when lentinan was administered prior to chemotherapy and in patients with a basis of prolongation of life, regression of tumors or lesions, and the improvement of immune responses.

According to Ref.[44], lentinan was injected into malignant peritoneal and/or pleural effusions of a group of 16 patients with advanced cancer. Eighty percent of the lesions showed probable clinical responses, with an improvement in performance demonstrated in seven subjects. The survival time for those who responded immunologically to the treatment was 129 days and 45 days for those who did not respond.

Shiitake mushrooms have cancer-preventative properties and can be a beneficial dietary supplement. Compounds that block the formation of carcinogenic *N*-nitroso compounds from nitrates (which occur in vegetables and meats) are produced in dried and heated mushrooms.[7,8,10] The uncooked form contains no detectable amounts of the nitrite-scavenging compound thiazolidine-4-carboxylic acid, while the dried variety has 134 mg/100 g (dry weight basis) of this compound, and the boiled form holds 850 mg/100 g.

In vitro studies have indicated that LEM from shiitake mushroom may be more effective than AZT in the treatment of AIDS (see discussions in the section on ''Preclinical studies''), because it inhibits the cytopathic effect of giant cell formation in a cell-free system with MT-4 cells, or a cell-to-cell infection system with MOLT-4 cells, both of which induce multinucleated giant cells very efficiently. *L. edodes* mycelium may work by blocking the initial stages of HIV infection.[39] Azidothymidine inhibits cell-free infection of HIV, but it is ineffective in preventing the formation of multinucleated giant cells. It is also expensive and is known to cause severe bone marrow toxicity and a host of other side effects. Furthermore, it may become less effective during long-term use or may not offer any long-term survival advantages even with early use. Mycelium extract, however, is nontoxic and less expensive. Its encapsulated form is recommended as a daily dietary supplement primarily for prevention of disease and maintenance of health. It must be stressed that more clinical trials will be necessary to assess the long-term benefit of the extract for HIV and AIDS. Another use is to boost the immune response in AIDS patients.[7,10,18] When it was used to treat HIV-positive patients with AIDS symptoms, the T-cell count rose from a baseline of $1250/mm^3$ after 30 days up to $2550/mm^3$ after 60 days. An improvement in clinical symptoms was also noted.

Lentinan has shown favorable results in treating chronic persistent hepatitis and viral hepatitis B.[10] Forty patients with chronic viral hepatitis B and seropositive for Hbe antigenemia were given 6 g of LEM daily (orally) for 4 mo. The study focused on the number of patients seroconverting from Hbe antigen positive to antiHbe positive, which was 25% after LEM therapy, and was higher in patients with chronic active hepatitis (36.8%). In addition, 17 patients (43%) became seronegative for Hbe antigen. Liver function tests improved even for patients who remained seropositive, and they had raised plasma albumin, and adjusted protein metabolism.

Dried shiitake mushroom (9 g/day) decreased 7–10% serum cholesterol in patients suffering with hypercholesterolemia. For many patients 60 years of age or older with hyperlipidemia, consuming fresh shiitake mushroom (90 g/day in 7 days) led to a decrease in total cholesterol blood level by 9–12% and triglyceride level by 6–7%.[10,36] Lentin, a novel protein isolated from shiitake mushroom, exerted an inhibitory activity on HIV-1 reverse transcriptase and proliferation of leukemia cells.[27,28]

Antifungal Activity

From the fruiting bodies of the shiitake mushroom, a novel protein designated lentin with potent antifungal activity was isolated in 2003.[28] It was unadsorbed on DEAE-cellulose, and adsorbed on Affi-gel blue gel and Mono S. The N-terminal sequence of the protein manifested similarity to endoglucanase. Lentin, which had a molecular mass of 27.5 kDa, inhibited mycelia growth in a variety of fungal species including *Physalosporia piricola, Botrytis cinerea*, and *Mycosphaerella arachidicola*.[28]

Toxicity and Side Effects

Shiitake mushroom is edible, but some individuals may experience minor side effects or allergic reactions.

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Literature describes[7,10,18,45] cases of shiitake-induced toxicodermia and shiitake dermatitis. Allergic reactions to the spores of shiitake mushrooms have been reported in workers picking mushrooms indoors, who are prone to an immune reaction to spores called ''mushroom worker's lung.'' Symptoms include fever, headache, congestion, coughing, sneezing, nausea, and general malaise.[46] A water extract of the fruiting body was found[47] to decrease the effectiveness of blood platelets in initiating coagulation. So people who bleed easily or who take blood thinners should be closely monitored when under long-term treatment with shiitake mushroom or its water-soluble fractions.

L. edodes mycelium has shown no evidence of being acutely toxic, even in massive doses of over 50 mg/day for 1 week, though mild side effects such as diarrhea and skin rash may occur.[a] As a rule, symptoms disappear after a short period, when the body has adapted to the extract. Lentinan has no known serious side effects. However, in clinical trials of patients with advanced cancer, minor side reactions occurred such as a slight increase in glutamate-oxaloacetate transminase (GOT) and GPT liver enzymes and a feeling of mild pressure on the chest. But these changes disappeared after lentinan administration was stopped.[34]

COMMERCIAL PREPARATIONS OF SHIITAKE MUSHROOMS

Dosage and Preparation

Shiitake mushroom is prescribed in various forms. It may be injected as a solution (1 mg/vial) or ingested as a sugar-coated tablet, capsule, concentrate, powdered extract, syrup, tea, wine, and/or as a medicinal dish. Lentinan's anticancer effect is highly dose-dependent. The standard dose of the dried fruiting body in tea or in mushroom dishes is given as 6–16 g, equivalent to about 90 g of fresh fruiting body. As a tablet, the dosage is usually in the form of 2 g tablets 2–4×/day.

Commercial preparations can be found in many countries in health food stores and supermarkets. The tablets are usually made from a dried water-extract of the mycelia or fruiting bodies because drying concentrates the lentinan and other active principals. Standardized extracts are also available, and they are preferred because the amount of lentinan present is certified and clearly stated on the bottle.

Although the fresh form can be a valuable dietary supplement, the quantities one would require for therapeutic doses are so great that its consumption could cause digestive upset. That is why LEM, which is concentrated and easily absorbed, is preferred for medicinal use.[7,8,10]

Fresh and dried shiitake mushrooms are used in medicinal mushroom dishes (''Yakuzen''). Certain medicinal effects have been recently studied[16] and found to reduce the ill effects of certain gourmet diets. These dishes can be prepared in many ways, limited only by one's ingenuity: boiled, grilled, skewered, or on aluminum foil with different types of seasoning. To obtain a concentrate, whole fruit bodies or powdered mushrooms are boiled in water. The extract is then concentrated, and is used as a drink. It can also be consumed as a tea: canned ''shiitake tea,'' which contains a concentrated extract, or many other shiitake ''healthy tea'' products sold as mushroom containing tea bags.

Shiitake mushroom concentrate can be freeze-dried or spray-dried to form a granular powder. There are many products containing powdered shiitake mushroom extract, such as a mixture of this powder with vitamin C crystals or with medicinal plants such as ginseng. In Eastern countries, the mushroom is mainly used as a concentrate when extracted with boiling water. Residues from these processes still contain substantial amounts of useful polysaccharide substances, including those effective as antitumor compounds such as β-glucans, nucleic acids, dietary fiber, etc.

An alcohol extraction product is obtained by preserving fresh or dried shiitake mushroom in alcohol, which has been mixed with sugar or molasses. Some products, including ''healthy shiitake wine,'' are sold as a nightcap or as a tonic drink.[8,9]

Drug Interactions

A watery extract of the whole fruiting body of *L. edodes* is reported to lessen the effectiveness of the blood platelets during the process of coagulation. People who bleed easily or who take blood thinners should use caution when chronically using *L. edodes* extracts in therapeutic amounts or in its water-soluble fractions (LEM).[10,47]

For cancer patients, smaller doses of intravenous and intramuscular lentinan are more effective than larger ones (e.g., 1 mg per injection is considered safe, whereas 10 mg may produce marked depression in the host immune response). Aoki[34] notes that what is considered an excessive dosage intravenously may be a favorable dosage when using oral administration.

For treating the initial stages of AIDS or chronic hepatitis, the best oral dose of LEM is between 2 and 6 g/day in 2–3 divided doses. If the disease is stable, the dosage may be decreased to 0.5–1 g/day.[7,10]

[a]The author does not consider these massive doses.

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