**Lentinus edodes**: A Macrofungus with Pharmacological Activities

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Abstract: *Lentinus edodes* is the first medicinal macrofungus to enter the realm of modern biotechnology. It is the second most popular edible mushroom in the global market which is attributed not only to its nutritional value but also to possible potential for therapeutic applications. *Lentinus edodes* is used medicinally for diseases involving depressed immune function (including AIDS), cancer, environmental allergies, fungal infection, frequent flu and colds, bronchial inflammation, heart disease, hyperlipidemia (including high blood cholesterol), hypertension, infectious disease, diabetes, hepatitis and regulating urinary inconsistencies. It is the source of several well-studied preparations with proven pharmacological properties, especially the polysaccharide lentinan, eritadenine, shiitake mushroom mycelium, and culture media extracts (LEM, LAP and KS-2). Antibiotic, anti-carcinogenic and antiviral compounds have been isolated intracellularly (fruiting body and mycelia) and extracellularly (culture media). Some of these substances were lentinan, lectins and eritadenine. The aim of this review is to discuss the therapeutic applications of this macrofungus. The potential of this macrofungus is unquestionable in the most important areas of applied biotechnology.

Keywords: Anti-cancer, eritadenine, immunomodulation, lentinan, *Lentinus edodes*, polysaccharide.

1. INTRODUCTION

Synthetic medicines (allopathic) after the Second World War completely outclassed the use of herbal folk medicine but during the last 20 years herbal products have re-emerged as a major competitor of synthetic products, being used in the form of herbal preparations, herbal teas, confectionery and non-alcoholic beverages [1]. Historically, higher fungi have long been used for medicinal purposes. Various important pharmaceutical products with proven medicinal applications have been derived from higher Basidiomycetes fungi [2]. *Lentinus edodes* (shiitake) is one of the world’s second largest cultivated medicinal and edible mushroom used as ‘Functional foods’ has a long history in oriental folklore for treatment of tumors, flu, heart diseases, high blood pressure, obesity, problems related to sexual dysfunction and ageing, diabetes, liver ailments, respiratory diseases, exhaustion and weakness. *L. edodes* is among the most valuable medicinal mushroom [3].

It is a source of two well-studied and widely approved polysaccharide medicines: LEM (an acronym for *L. edodes* mycelia), a protein-bound polysaccharide derived only from the mycelium [4], and lentinan – a cell-wall branched – β-D-glucan extracted from both the fruiting body and mycelium [5]. Both compounds are immune system enhancers that demonstrated anticancer activity. Additionally, *L. edodes* contains other compounds that inhibit blood aggregation; reduce cholesterol levels, and exhibit antibacterial and antiviral effect [6, 7]. A lignan-rich compound derived from *L. edodes* mycelium holds a promising factor for treating both hepatitis-B and AIDS [8]. Another anti-tumour active polysaccharide, KS-2, has been isolated from *L. edodes* mycelia (LEM) [9]. It is a good source of carbohydrate, protein and essential amino acids. It is low in fat, have a high percentage of polyunsaturated fatty acids and also contain many vitamins and minerals. The protein in *L. edodes* are composed of 18 types of amino acids, including all essential amino acids in ratios similar to that ideal for human nutrition [10]. *L. edodes* contains significant quantities of vitamins C, B1, B2, B12, niacin, and vitamin D (the highest content of vitamin D of any plant food) [11]. *L. edodes* mushroom-derived polysaccharides, immunomodulating and anticancer compounds are used in clinical applications as adjuvant to standard chemotherapy. Another potential use for *L. edodes* is as foodstuff, consumed whole or in concentrated extract or as dietary supplement [12]. There are several types of dietary supplements and medicinal formulations derived from *L. edodes*: dried and pulverized fruiting bodies, hot water and alcohol extracts of fruiting bodies, biomass or extracts of mycelia, or broth harvested from submerged liquid cultures. Commercial preparations are available as tablets, capsules, or elixirs, and are available in most Asian countries and are increasingly available in USA, New Zealand, Australia, and Europe.

2. THE MACROFUNGUS

2.1. Habit and Habitat

*L. edodes* belongs to the kingdom-Fungi, Phylum-Basidiomycota, Class-Basidiomycetes, order-Agaricales, family-Agaricaceae, genus-*Lentinus* and species-*edodes* [13]. It produces sexual basidiospores, which are the reproductive units of this fungus. The fruit bodies (basidiocarps) contain the basidiocarp-producing structures, basidia [14]. Each basidium carries four basidiospores, and each basidiospore in turn contains one haploid nucleus. Once basidiospores have been released and the conditions are favourable they germinate to form hypha, a thread like fungal cell, further divided into hyphal compartments. The hypha is surrounded by a cell wall mainly composed of chitin and glucans (polymers of glucose) having a single nucleus in each
compartment (monokaryon). When the hyphae of two different mating compatibility groups fuse with one another a dikaryotic hyphae are produced, containing two nuclei in each hyphal compartment (one from each compatibility group). The hyphae continue to grow by extension at the hyphal tip and branches repeatedly to form hyphal filaments in a complex network, mycelium, which is the vegetative part of the fungus. The mycelium of the fungus is responsible for the capturing of nutrients for growth and is often hidden underground or in the decaying organic material.

When the surrounding conditions are right, mycelia start to form a fruit body completing one life-cycle of the mushroom (Fig. 1). All the tissues of the fruit body are composed of dikaryotic hyphae, and within each basidium the two nuclei are fused to produce a diploid nucleus [14]. The subsequent meiosis produces haploid nuclei, which then migrate into the developing basidiospores, one in each of the four basidiospores. The basidiospores reside in the basidium until release and the beginning of a new life cycle of the mushroom.

Lentinus edodes is a wood-decaying basidiomycetes, 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 in a warm, moist climate [15]. The geographic distribution of shiitake in nature extends beyond northeast Asia, but the exact limits are uncertain. The minimum range, based on reliable, well documented reports, extends from northern Japan as far south and east as Tasmania and New Zealand and as far west as the Himalayan regions of Bhutan, Nepal, and India [16, 17]. Samgina [18] reported that shiitake occurs in Kazakhstan, where the mushrooms were said to have been growing on conifer wood. However, shiitake is typically known only to occur on logs of broadleaf trees, especially Fagaceae [19, 20], which suggests the fungi in Kazakhstan may have been misidentified.

The distribution of shiitake in the Pacific islands north and east of Papua New Guinea, such as New Caledonia, is also poorly documented.

Pegler [16] correlated morphological differences with geographic distribution to recognize three species of shiitake: Lentinus edodes in northeast Asia, Lentinus lateritia in Southeast Asia and Australasia, and Lentinus nauseolandiae in New Zealand. Other mycologists have questioned whether Pegler’s species are in fact morphologically distinct [17]. Pegler’s species concept have also been challenged on the basis of mating criteria. Isolates of shiitake from Japan, Papua New Guinea, Borneo, Nepal, and New Zealand have shown to be mating compatible in laboratory crosses [17], which has led some mycologists to recognize only one species of shiitake Lentinus edodes.

3. CHEMICAL CONSTITUENTS AND BIOACTIVE COMPONENTS OF L. EDODES

Scientific investigations have led to isolation of many compounds from L. edodes having health promotion activities [15, 21, 22]. Fruit bodies of L. edodes contains 88–92% water, protein, lipids, carbohydrates as well as vitamins and minerals (Table 1) [23]. The mushroom is a good source of vitamins (Table 2), especially provitamin D2 (ergosterol),

Fig. (1). Different Growth stages of Lentinus edodes during artificial cultivation; A, Culture plate (Growing in Potato Dextrose Agar Medium); B, Spawn (Prepared in wheat grain); C, Mycelial colonization on solid substrate; D, Bump and primordia formation; E, Maturation of fruitbody; F, Mature fruit body.
which under ultraviolet (UV) light and heat yields calciferol (vitamin D2). It also contains B vitamins, including B1 (thiamine), B2 (riboflavin) and B12 (niacin) [21, 22, 24-26]. The fatty acids account for 3.38% (Table 3) of the total lipids [21,27] with an appreciable amount of amino acids (Table 4) [23].

In addition to glycogen-like polysaccharides, (1-4)-, (1-6)-β-D-glucans and antitumor polysaccharides, lentinan, (1-3)-, (1-6)-β-bonded heteroglucans, heterogalactans, heteromannans, xyloglucans, etc., have been identified. Among the free sugars present are trehalose, glycerol, mannitol, arabinitol, mannose, and arabinose [15, 21, 22, 24]. 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 polyuronic (acidic polysaccharide), hemicellulose, β-glucan with heterosaccharide chains, lignin, and chitin present as cell wall constituents. The aroma components include alcohols, ketones, sulfides, alkanes, fatty acids, etc [21]. 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 [21], the components responsible for the delicious flavor are monosodium gluta-

Table 1. Proximate and Mineral Composition of Lentinus edodes (per 100 gm Sample) [23]

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Components</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Moisture</td>
<td>4.7gm</td>
</tr>
<tr>
<td>2</td>
<td>Protein</td>
<td>22.8gm</td>
</tr>
<tr>
<td>3</td>
<td>Fat</td>
<td>2.1gm</td>
</tr>
<tr>
<td>4</td>
<td>Ash</td>
<td>6.0gm</td>
</tr>
<tr>
<td>5</td>
<td>Carbohydrate and Fibre</td>
<td>64.4gm</td>
</tr>
<tr>
<td>6</td>
<td>Energy (Kcal)</td>
<td>411</td>
</tr>
<tr>
<td>7</td>
<td>Phosphorus</td>
<td>439mg</td>
</tr>
<tr>
<td>8</td>
<td>Magnesium</td>
<td>200mg</td>
</tr>
<tr>
<td>9</td>
<td>Calcium</td>
<td>127mg</td>
</tr>
<tr>
<td>10</td>
<td>Iron</td>
<td>20.1mg</td>
</tr>
<tr>
<td>11</td>
<td>Zinc</td>
<td>4.3mg</td>
</tr>
<tr>
<td>12</td>
<td>Manganese</td>
<td>5.1mg</td>
</tr>
<tr>
<td>13</td>
<td>Copper</td>
<td>0.9mg</td>
</tr>
<tr>
<td>14</td>
<td>Chromium</td>
<td>140 μg</td>
</tr>
</tbody>
</table>

Values are expressed on dry weight basis.

Table 2. Vitamin Composition of Lentinus edodes (Quantities Per 100g) [25, 26]

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Vitamins</th>
<th>Concentration</th>
<th>fresh</th>
<th>dry</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Thiamin</td>
<td>0.05mg</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Riboflavin</td>
<td>0.15mg</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Niacin</td>
<td>2.6mg</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Ascorbic acid</td>
<td>2.1mg</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Vitamin K</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Pantothenic acid</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Biotin</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Pyridoxin</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Folic acid</td>
<td>0.03mg</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Vitamin D</td>
<td>-</td>
<td>4000 i.u.</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Pro-Vitamin D Ergosterol</td>
<td>679μg</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Fatty Acid Composition of Lentinus edodes (Values Are Percent of Total Fat) [23]

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Fatty acid</th>
<th>Concentration in %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C 16.0 Palmitic</td>
<td>19.2</td>
</tr>
<tr>
<td>2</td>
<td>C 18.0 Stearic</td>
<td>2.7</td>
</tr>
<tr>
<td>3</td>
<td>C 20.0 Arachidic</td>
<td>0.4</td>
</tr>
<tr>
<td>4</td>
<td>C 18.1 Oleic</td>
<td>8.3</td>
</tr>
<tr>
<td>5</td>
<td>C 18.2 Linoleic</td>
<td>68.8</td>
</tr>
<tr>
<td>6</td>
<td>C 18.0 Linolenic</td>
<td>0.6</td>
</tr>
<tr>
<td>7</td>
<td>Total Saturates</td>
<td>22.3</td>
</tr>
<tr>
<td>8</td>
<td>Total Unsaturates</td>
<td>77.7</td>
</tr>
</tbody>
</table>

Table 4. Amino Acid Composition of Lentinus edodes [23]

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Amino acid</th>
<th>Concentration (g16gN)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Threonine</td>
<td>3.2</td>
</tr>
<tr>
<td>2</td>
<td>Valine</td>
<td>6.7</td>
</tr>
<tr>
<td>3</td>
<td>Cysteine</td>
<td>1.4</td>
</tr>
<tr>
<td>4</td>
<td>Methionine</td>
<td>0.8</td>
</tr>
<tr>
<td>5</td>
<td>Isoleucine</td>
<td>4.9</td>
</tr>
<tr>
<td>6</td>
<td>Leucine</td>
<td>7.3</td>
</tr>
<tr>
<td>7</td>
<td>Tyrosine</td>
<td>3.3</td>
</tr>
<tr>
<td>8</td>
<td>Phenylalanine</td>
<td>4.2</td>
</tr>
<tr>
<td>9</td>
<td>Lysine</td>
<td>6.4</td>
</tr>
<tr>
<td>10</td>
<td>Histidine</td>
<td>2.3</td>
</tr>
<tr>
<td>11</td>
<td>Arginine</td>
<td>8.0</td>
</tr>
<tr>
<td>12</td>
<td>Aspartate</td>
<td>9.9</td>
</tr>
<tr>
<td>13</td>
<td>Serine</td>
<td>5.3</td>
</tr>
<tr>
<td>14</td>
<td>Glutamate</td>
<td>12.6</td>
</tr>
<tr>
<td>15</td>
<td>Proline</td>
<td>8.0</td>
</tr>
<tr>
<td>16</td>
<td>Glycine</td>
<td>5.1</td>
</tr>
<tr>
<td>17</td>
<td>Alanine</td>
<td>7.8</td>
</tr>
<tr>
<td>18</td>
<td>Total essential amino acids</td>
<td>38.2</td>
</tr>
<tr>
<td>19</td>
<td>Total amino acids</td>
<td>97.2</td>
</tr>
<tr>
<td>20</td>
<td>% Essential amino acids</td>
<td>39</td>
</tr>
<tr>
<td>21</td>
<td>Chemical score</td>
<td>29</td>
</tr>
</tbody>
</table>
mate, 50-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, -keto-glutaric acid, oxalic acid, lactic acid, acetic acid, formic acid, and glycolic acid.

3.1. Lentinan

Lentinan is a high molecular weight \(5 \times 10^5\) Da; \([\alpha] D +20–22^\circ\) (NaOH) polysaccharide \([(C_{6}H_{10}O_{5})_n]\) extracted from cell wall of fruiting body in a triple helix structure containing only glucose molecules with mostly \(\beta-(1-3)\)-glucose linkages in the regularly branched backbone, and \(\beta-(1-6)\)-glucose side chains (Fig. 2) [15, 21, 28]. The configuration of the glucose molecule in a helix structure is considered to be important for the biological and pharmacological activity [29]. Lentinan is completely free of any nitrogen (and thus protein), phosphorus, sulphur and any other atoms except carbon, oxygen and hydrogen. It is water soluble, heat stable and alkali labile.

3.2. LEM and LAP (EP3)

LEM and LAP extracts are derived from \(L. edodes\) mushroom mycelium and culture media respectively. Both are glycoproteins containing glucose, galactose, xylose, arabinose, mannose and fructose. LEM also contains nucleic acid derivatives, vitamin B compounds and ergosterol. LEM and LAP both demonstrated strong antitumour activity by i.p., and p.o. in animals and humans. LEM is prepared from a hot water extract of powdered mycelia, incubated for 50-60 h at 40-50°C and partially hydrolysed by endogenous enzymes. The residue was extracted with water, 60°C, and the filtrate freeze dried. The light brown powder was LEM. An immunoactive substance EP3 has been obtained by further fractionation of LEM. [4, 22].

3.3. KS-2-\(\alpha\)- Mannan Peptide

KS-2 is a peptide–polysaccharide complex (MW 6–9.5 \times 10^4 \([\alpha] D +62^\circ\); C = 0.5, water) which was obtained by extraction of cultured mycelia of \(L. edodes\) mushroom with hot water, followed by precipitation with ethanol [21, 27, 9]. It is an \(\alpha\) -mannan peptide containing the amino acids serine, threonine, alanine, and proline (as well as residual amounts of the other amino acids). The polysaccharide is shown to be effective on Sarcoma 180 and Ehrlich’s carcinoma, either i.p. or p.o., and to act via interferon-inducing activity [9].

3.4. Eritadenine

Eritadenine was isolated from \(L. edodes\) mushroom for the first time and identified as the active cholesterol lowering component [30]. Eritadenine, \(2(R), 3(R)\)-dihydroxy-4-(9-adenyl) - butyric acid, was formerly designated as lentinacin [30] and lentsyline [31, 32] by the research groups who individually isolated and structurally determined this compound (Fig. 3). Eritadenine is a secondary metabolite produced mainly by \(L. edodes\). The ability of eritadenine is to lower the blood cholesterol level. It has been reported that eritadenine was present in \(L. edodes\) at levels of 400-700 mg/kg dry matter [33].

4. PHARMACOLOGICAL ASPECTS OF \(L. edodes\)

\(L. edodes\) is one of the well-known macrofungus used in several therapeutic applications. It is the source of several well-studied preparations with proven pharmacological properties. The medicinal properties of \(L. edodes\) (Fig. 4) have been studied since Ming Dynasty (1369-1644) [34]. The elders from Japanese Empire considered shiitake as the “elixir of the life” increasing vigor and energy [35]. Antibiotic, anti-carcinogenic, anticancer, antifungal, antibacterial and antiviral, antidiabetic, hypolipidemic compounds have been isolated intracellularly (fruiting body and mycelia) and extracellularly (culture media) from \(L. edodes\). Some of these substances were lentinan, lectin and eritadenine [30, 36-40]. The shiitake mushroom is used medicinally for diseases involving depressed immune function (including AIDS), cancer, diabetes, environmental allergies, fungal infection, frequent flu and colds, bronchial inflammation, and regulating urinary incontinence. A summary of the therapeutic effects and bioactive compounds of \(L. edodes\) which is reported in the literature till 2009 has been represented in Table 5. Major therapeutic effects of this wonderful mushroom is being elaborated below.

4.1. Antitumor and Anticarcinogenic Activity

The antitumor polysaccharide ‘Lentinan’ was first isolated and studied by Chihara et al. [41] who demonstrated that its anti-tumor effects were greater than other mushroom
polysaccharides and was active for some types of tumors [42]. The antitumor effect of lentinan was confirmed by using Sarcoma-180 transplanted in CD-1/ICD mice [5]. 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 [43]. The tumor inhibitory effect of lentinan was highly striking. In 1-5 mg/kg x 10 doses, the inhibition ratio was 95 to 97.5%, and in dosages of 0.2 mg/kg x 10, the tumors underwent complete regression in 6 out of 10 mice [41]. Combination treatment of L. edodes mycelium extracts with 5-Fluorouracil represent a novel chemotherapeutic strategy in colon cancers and that p53, p21/Cip1 and p27/Kip1 may play some important roles for the involvement in antitumor activity [44]. Four antitumor (1/3)-β-D-glucans coded as L-I1, L-I2, L-I3 and L-I4 with high molecular weight (1.47x10^6–1.67x10^6) were isolated from four kinds of fruiting bodies of Lentinus edodes [45]. Exo-biopolymer from rice bran cultured with L. edodes [rice bran exo-biopolymer (RBEP)] induced the activation of NK cells in a dose-dependent manner when administrated orally [46].

The carcinostatic effect of lentinan results from the activation of the host’s immune system. 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 [47]. The antitumor studies conducted with L. edodes thus far are very interesting and do show a potential for providing therapeutic control of cancer. It is, however, difficult to say whether L. edodes could have preventive effects against cancer when consumed as part of the diet.

4.2. Immunomodulating Effects

Lentinus edodes has attracted a lot of attention owing to its immunomodulatory effects. Lentinan is well known as a type of biological response modifier (BRM). Augmentations
of NK (Natural Killer), CTL (Cytotoxic T Lymphocyte), LAK (Lymphokine Activated Killer) activities and DTH (Delayed Type Hypersensitivity) responses against tumor antigen were observed after administration of Lentinan [48]. These activities are responsible for the antitumor effects of lentinan. Antitumor polysaccharide L-II was isolated and purified from the fruiting body of *L. edodes* [49].

The antitumor activity of the polysaccharide L-II on mice-transplanted sarcoma 180 was mediated by immunomodulation by inducing T-cells and macrophage-dependent immune system responses. Kupfahl *et al.* [50] evaluated *Listeria monocytogenes*. The results showed that the lentinan enhances the protective CD8 T-cell response against *L. monocytogenes* probably by a mechanism that involves the IL-12-mediated augmentation of the specific antilisterial CD8 T-cell response. Fruit body and mycelial extracts of *L. edodes* are able to enhance the proliferation of rat thymocytes directly and act as co-stimulators in the presence of the T-mitogen PHA [51]. Many interesting mechanisms of action of Lentinan and possible pathways for inflammatory and immune reactions have been represented in Fig. (5) [52].

Pre-treatment of mice with lentinan significantly decreased the parasitemia and increased their survival after infection by *Plasmodium yoelii* 17XL (P.y17XL). Enhanced IL-12, IFN-Γ and NO production induced by lentinan in spleen cells of infected mice revealed that the Th1 immune response was stimulated against malaria infection. *In vitro* and *in vivo*, lentinan enhanced the expression of MHC II, CD80/CD86, and Toll-like receptors (TLR2/TLR4), and increased the production of IL-12 in spleen dendritic cells (DCs) co-cultured with parasitized red blood cells (pRBCs). It was concluded that lentinan has prophylactic potential for the treatment of malaria [53]. The immunomodulation effects of lentinan through oral administration were investigated [54] and the results obtained proved its efficacy, that prefeeding of the mice for 7 days at an optimum dose of 3 mg/mouse was most effective against tumor induction, achieving a tumor inhibition rate (TIR) of 94.44%. Possible immune system regulating actions of lentinan were summarized [43] and are depicted in Fig. (6).

### 4.3. Antimicrobial Activity

Antimicrobial activity has been found in liquid cultures, chloroform, ethyl acetate, water and dried fruit body extracts of *L. edodes* [55-57]. These extracts are active against gram positive and gram-negative bacteria, yeasts and mycelial fungi, including dermatophytes and phytopathogens [58]. Mycelial-free culture of *L. edodes* exhibited greater antimicrobial effect against grampositive than gram-negative bacteria with *Bacillus subtilis* and *Staphylococcus aureus* among the most highly inhibited [56]. Antimicrobial compounds isolated from *L. edodes* liquid cultures include lentinamicin.
4.3.1. Antiviral Activity

*L. edodes* had been believed to cure the common cold for hundreds of years. More recently, some scientific evidences have been obtained to support this belief. *L. edodes* showed an activity (expressed as the percentage decrease in lung lesion score compared with the control) of 46%, which was of the same magnitude as for amantadine hydrochloride, a common drug against influenza (40%). A watery extract from *L. edodes* was also reported to prevent the multiplication of polio virus [59]. Lentinan enhanced the host resistance against infections with bacteria, fungi, parasites, and viruses, including the agents of AIDS [60]. Lentinan reduced the toxicity of azidothymidine AZT (a drug commonly used for treating HIV carriers and AIDS patients). Prevention of the onset of AIDS symptoms through potentiation of host defense is now being actively investigated both experimentally and clinically [61]. In addition to lentinan, other substances from *L. edodes* have also been shown to have antiviral activity. The mechanism of their effect is in most cases via induction of interferon [3, 21].

Lentinan has also shown: (a) antiviral activity in mice against VSV (vesicular stomatis virus), encephalitis virus, Abelson virus, an adenovirus type 12; (b) stimulated nonspecific resistance against respiratory viral infection in mice; (c) conferred complete protection against an LD75 challenge dose of virulent mouse influenza A/SW15; (d) increased resistance to the protozoal parasites *Schistosoma japonicum*, *Sch. mansoni*; (e) exhibited activity against *Mycobacterium tuberculosis* bacilli resistant to antituberculosis drugs, *Bacillus subtilis*, *Staphylococcus aureus*, *Micrococcus lenteus*, *Candida albicans* and *Saccharomyces cerevisiae*; (f) increased host resistance to infections with potentially lethal *Listeria monocytogenes* [2].

LEM and a new lignan-rich compound ‘JLS-18’ derived from LEM, blocked the release of infectious *Herpes simplex* virus in animals [62] and it has been suggested because of its high activity that JLS-18 could be of value in the treatment of hepatitis B and AIDS patients [63]. Water-soluble lignins from EP3 and EPS4 from shiitake mushroom mycelium have shown antiviral effects [64].

4.3.2. Antibacterial Activity

Anti-bacterial activity is an exciting result, with increasing bacterial resistance to antibiotics, improving host immunity may be the way forward in fighting bacterial infection. The antibacterial activity of *L. edodes* against *Bacillus subtilis* was evaluated in cell-free filtrates obtained after growth in 14 different culture media [65]. Lentinonine, a sulphur containing peptide from shiitake has antibacterial and antifungal activity and bis (methylsulfonyl) methyl disulphide [66], a derivative of lentinonine, has strong inhibitory effects against *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli* [67]. The chloroform and ethylacetate extracts of the dried shiitake mushroom have bactericidal activity against both growing and resting *Streptococcus mutans* and *Prevotella intermedia* [68].

4.3.3. Antifungal Activity

A novel protein designated lentin with potent antifungal activity was isolated by Ngai and Ng [69] from the fruiting bodies of the *L. edodes*. Lentin, which had a molecular mass of 27.5 kDa, inhibited mycelial growth in a variety of fungal species including *Physalospora piricola*, *Botrytis cinerea*...
and *Mycosphaerella arachidicola*. Lentin also exerted an inhibitory action on HIV-1 reverse transcriptase and proliferation of leukemia cells.

### 4.4. Cardiovascular and Hypolipidemic Activity

Cardiovascular diseases are among the main causes of death in our society and there is a strong correlation between enhanced blood cholesterol levels and the development of such diseases. The popular macrofungus *L. edodes*, has been shown to produce blood cholesterol lowering compound designated eritadenine [2(R), 3(R)-dihydroxy-4-(9-adenyl)butyric acid] [30]. The hypocholesterolemic action of this compound has been quite extensively examined in rats. Eritadenine is suggested to accelerate the removal of blood cholesterol either by stimulating tissue uptake or by inhibiting tissue release [70]. It was demonstrated that when rats were fed a diet supplemented with 5% (dry weight) of *L. edodes* fruiting bodies for 10 weeks the plasma cholesterol levels of the animals decreased significantly [71]. Eritadenine works by lowering levels of all lipoprotein types, i.e., high-density as well as low-density lipoproteins [3].

In addition to animal tests, the effectiveness of *L. edodes* in lowering blood serum cholesterol was also tested in human subjects. A daily intake of 90 g of fresh shiitake, 9 g of dried shiitake, and 9 g of UV-irradiated dried shiitake for 7 days lowered the mean serum cholesterol levels in young women by 12, 7 and 6%, respectively [7]. All three diets decreased serum cholesterol levels of older persons (60 years of age) by 9% over 7 days. Eritadenine reduces blood serum cholesterol 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 [6, 22, 24]. The amount of cholesterol reducing agent (eritadenine) in *L. edodes*, in search of a potential natural medicine against blood cholesterol was quantified [70, 72].

#### 4.5. Antidiabetic Activity

The hypoglycemic effect of an exo-polymer produced from a submerged mycelium culture of *L. edodes* was investigated in streptozotocin-induced diabetic rats [6], the administration of the exo-polymer (200 mg/kg BW) reduced the plasma glucose level by as much as 21.5%, and increased plasma insulin by 22.1% as compared to the control group. It also lowered the plasma total cholesterol and triglyceride levels by 25.1 and 44.5%, respectively. The hypoglycemic effect of *L. edodes* has been also demonstrated and proved its potential in lowering the blood glucose and triglyceride (TG) levels in the serum of rats [73]. It also lowered the plasma total cholesterol and triglyceride levels by 25.1 and 44.5%, respectively.

Exopolysaccharide (EPS) produced from submerged mycelial culture of *Lentinus* species was evaluated for hypoglycemic activity in streptozotocin (STZ)-induced diabetic rats [74]. In dose-dependent study, orally administered *L. strigosus* EPS, at the dose of 150 mg/kg, exhibited a considerable hypoglycemic effect in STZ-induced diabetic rats. Plasma insulin levels of STZ-induced diabetic rats decreased as compared to control group rats. Although insulin levels slightly increased in the EPS-treated groups. The hypoglycemic potential of the EPS was further supported by histological observations of pancreatic islets of Langerhans.

#### 4.6. Hepatoprotective Activity

A polysaccharide fraction from *L. edodes* showed liver protective action in animals together with improved liver function and an enhanced production of antibodies to hepatitis B [21]. Lentinan and LEM have given favourable results in treating chronic persistent hepatitis and viral hepatitis B patients [75]. 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 [2, 22, 27].

The hot-water extraction and ethanol extraction of the mycelia of *L. edodes* were examined for their hepatoprotective effect on dimethylaminosamine-injured mice [76]. Both fractions decreased the blood aspartate aminotransferase and alanine aminotransferase levels, partially inhibited the over accumulation of collagen fibrils, and suppressed the over expression of genes for alpha-smooth muscle actin and/or heat-shock protein 47 in the mice. Both fractions also inhibited the morphologic change and proliferation of isolated rat hepatic stellate cells (HSCs), which play a central role in liver fibrosis, in a dose-dependent manner and without cytotoxicity. The direct interaction between the extracts and HSCs appears to be important for the hepatoprotective activity. Polyphenols contained in both fractions are considered to be potential candidates for expressing the hepatoprotective effects.

#### 4.7. Hemagglutinating Activity

The agglutinating activity of different morphogenetic structures of *L. edodes* F-249, including mycelium, brown mycelial mat, primordia, and fruiting bodies was studied. Data showed that mycelial mat was found to possess the maximum hemagglutinating activity, which can be explained by the possible involvement of agglutinins in the formation of mycelial mat, which is composed of glued hyphae. The changes of the hemagglutinating activity of intracellular lectins of the basidiomycete *L. edodes* at various stages of its morphogenetic development depending on erythrocyte type, growth medium, and lectin purification degree was studied [77-79].

#### 4.8. Antioxidant Activity

An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals, which start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing...
free radical intermediates, and inhibit other oxidation reactions by being oxidized themselves.

Pharmacological effect of polysaccharides from *L. edodes* on serum oxidative status in high-fat-diet rats were investigated [80]. The rats were fed a standard diet and had free access to water. The administration of polysaccharides from *L. edodes* significantly reduced serum total cholesterol (TC), triglyceride (TG), low density lipoprotein cholesterol (LDL-c) and enhance serum antioxidant enzyme activity and thymus and liver index in high-fat rats. In addition, the administration of polysaccharides from *L. edodes* significantly decreased the increased expression level of VCAM-1 mRNA. In conclusion, the data suggested that the administration of polysaccharides from *L. edodes* could decrease the increased oxidative stress induced by high-fat diet in rats.

The antioxidant activity of *L. edodes* extracts obtained by organic solvents and supercritical fluids using different extraction techniques; high-pressure operations and low-pressure methods were investigated [81]. The mushroom extract was mixed with a 0.3 mM 2, 2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) ethanol solution, to give final concentrations of 5, 10, 25, 50 and 100 μg of extract per mL of DPPH solution. Fractions obtained with CO2 using ethanol as co-solvent, at 40ºC, 20 MPa and 15% EtOH and for dichloromethane in low-pressure technique had antioxidant activities.

The effect of heat treatment on the changes in the overall antioxidant activity and polyphenolic compounds of *L. edodes* investigated by Choi et al. [82]. The heat treatment significantly enhanced the overall antioxidant activities of shiitake mushroom. The low molecular weight (LMW) subfraction of the water extract of *L. edodes* had the highest antioxidant activity against lipid peroxidation of rat brain homogenate with IC50 values of 0.109 and 1.05 mg/ml [83]. Bioprocessing was very effective in mobilizing phenolic antioxidants from soybean powder. Post processing, phenolic content had increased 90% to 11.3 mg/g dry weight (wt) in water extract and 232% to 5.8 mg/g dry wt in ethanol extract [84]. Hence, it was clearly concluded that *L. edodes* had a power-pack antioxidant property which would be beneficial for mankind.

5. CLINICAL STUDIES OF *L. EDODES* IN HUMANS

Lentinan was demonstrated to have antitumor activity and increased the survival time of patients with inoperable gastric cancer [22] and women with recurrent breast cancer following surgical therapy [15, 22]. When the polysaccharide of *L. edodes* was 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 [85]. 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. 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. Lentinan was injected into malignant peri-
toneal and/or pleural effusions of a group of 16 patients with advanced cancer [86]. 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.

Mycelium extract of *L. edodes* was used to boost the immune response in AIDS patients [15, 22]. When it was used to treat HIV-positive patients with AIDS symptoms, the T-cell count rose from a baseline of 1250/mm3 after 30 days up to 2550/mm3 after 60 days. Forty patients with chronic viral hepatitis B and seropositive for Hbe antigenemia were given 6 g of LEM daily (orally) for 4 months. 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 from 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% [22, 6].

6. TOXICITY AND SIDE EFFECTS OF *L. EDODES*

*Lentinus edodes* is an edible mushroom, but some individuals may experience minor side effects or allergic reactions. Allergic reactions to the spores of *L. edodes* have been reported in workers picking mushrooms indoors. Symptoms include fever, headache, congestion, coughing, sneezing, nausea, and general malaise [87]. A water extract of the fruiting body was found to decrease the effectiveness of blood platelets in initiating coagulation [88]. *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.

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 [89].

7. COMMERCIAL ASPECTS OF *L. EDODES*

Today, present need in every aspect has increased so fast that every research work with commercial outlook requires major modifications to meet the upcoming challenges and obstructions. Similarly, the present review is an attempt to create an understanding and make aware about the vital importance of the macrofungus "*Lentinus edodes*". The great interest in *L. edodes* commercialization is due to its flavor/taste, and several medicinal applications. More than 130,000 tons of *L. edodes* are produced per year, out of them 45% of this is sold fresh, the rest being sold dried. *L. edodes* is commercially available in many 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. The therapeutic uses of *L. edodes* for the treatment of a variety of diseases, including, AIDS/HIV support, cancer, fatigue, hepatitis, high cholesterol, hypertension, immune support, intestinal parasites/worms, longevity, respiratory infections speak about *L. edodes*’s commercial significance.

### 8. CONCLUSION AND FUTURE PROSPECTS

The *Lentinus edodes* has been described in the literature as a macrofungus with great potential for therapeutic applications. Medicinal value of this mushroom has become a matter of great significance particularly in preventing or treating serious health conditions such as cancer, acquired immune deficiency syndrome (AIDS), and hypercholesterolemia. The potential of this macrofungus is unquestionable in some of the most important areas of applied biotechnology. However, there are currently no standard protocols for verifying the product quality and efficacy. From a pharmacological point of view, safety is the primary issue and research in this direction is desired. *L. edodes* has a great potential for the production of useful bioactive metabolites and that they serve as a prolific resource for drugs. The identified bioactive compounds in *L. edodes* of known molecular structures account for a wide range of beneficial biomedical effects, most notably in prevention of diverse physiological disorders and diseases. Further research is needed to establish content and bioactivity of the many compounds in human context.

Though there are many reports available for the cultivation of *L. edodes* the quality and content of physiologically active substances vary from strain to strain and also depend on location, culture conditions and growth of the mushroom. Its cultivation on solid substrates, stationary liquid medium or by submerged cultivation under different physiological and chemical conditions has become essential to meet the increasing demands in the international markets. Genetic engineering can also be used for development of potential strains. Overall it would seem that science supports the folklore. Many studies have demonstrated therapeutic effects of *L. edodes* although there is still much that is unknown and many more areas of its effects need exploration and thus the functional mushroom *L. edodes* deserve further serious investigation.

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