

Studies on Bioactive Substances and Medicinal Effects of REISHI, *Ganoderma lucidum* in Japan

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REISHI, the fruiting body of Mannentake, *Ganoderma lucidum* has been known in Japan, China, and other countries as a food and raw material for the development of drugs. Recent studies have shown that the carcinostatic substance in REISHI is a polysaccharide, β -8-(1 \rightarrow 3)-D-glucan. This polysaccharide seems to have promise as a new type of carcinostatic agent which might be useful in immunotherapy. Unlike chemicals used in chemotherapy, it has few toxic side effects because its effect is based on immunological enhancement in the host. REISHI also seems to contain other substances which reduce blood pressure, blood cholesterol, and blood sugar levels; and inhibit platelet aggregation, etc.

Recently, in vitro immunomodulating effects of Reishi extracts, and clinical study of micronized Reishi in Thai HIV and AIDS patients were attempted in Thailand. Some lanostane tri-terpenoids have been isolated from *G. lucidum*. These are highly oxidized compounds which show interesting biological activities.

1. Artificial Cultivation of REISHI

Artificial culture and cultivation of Mannentake, *G. lucidum* fungus were attempted initially by T. Henmi et al., in 1937. Its mass production was first achieved by Y. Naoi in 1971 by cultivating the spawn using pots containing sawdust. Since then use of bed logs or sawdust has become established practice. For some purposes a bagasse spawn bed method and bottle or bag cultivation may be used. Usually, for mass cultivation of high quality Reishi, either out doors, bed logs of white oak, oak, Japanese chest-nut, Japanese apricot, etc., are used.

The 1995 production of Reishi in Japan was estimated to be about 500 tones dry weight. Reishi cultivation has also prospered in China, Taiwan, Korea, Thailand, and Vietnam. In addition, attempts are being made to obtain useful cellular materials or to produce effective substances from cultured mycelia.

2. Pharmacologically Active Components in REISHI

2.1. General Chemical Components in REISHI

The major components and the free amino acids in Mannentake fruiting body (Reishi) have been determined. Both qualitative and quantitative differences are found in the composition of naturally grown and artificially cultivated sampled of Reishi, depending on the lines, places of the production, cultivation conditions, etc.

2.2. Medicinal Effects of REISHI Extract with Hot Water

The hot water extract (ca. 10% dry matter) obtained from Reishi seems to have some properties. Little could be done to isolate the pure substances having these medicinal properties until recently, when the culture and cultivation of Mannentake became possible.

The results of this research are described below. Antitumor substances whose properties have been studied in depth are described in detail in the article Antitumor-Active Substances from Mushrooms.

2.3. Bitter Terpenoids

The fruiting body of Mannentake (Reishi) is extremely bitter, a characteristic not found in any other mushroom. The bitterness varies in degree depending on the place of production, cultivation conditions, its strain, etc. This bitterness is not found in cultured mycelia or substances produced in the culture medium, and Kokushi (black Reishi) does not contain the bitter substance. Though the relationship between bitterness and pharmacological effects is not fully understood, the bitterness serves as a marker for pharmacological evaluation and classification of *Ganoderma* sp.

The bitter components and related compounds, highly oxidized lanostane triterpenoids, have been isolated from the

extracts of Reishi by various chromatographic procedures, and their bitterness was evaluated. Some other triterpenoids isolated from Mannentake have been studied for their anti-allergy action, etc.

2.4. Steroides

Ergosterol (provitamin D₂) has been reported in concentrations of 0.3-0.4% in Reishi. However, further analysis has confirmed that the main component of the steroids is 24-methylcholesta-7, 22-dien-3-,6-ol. Ergosterol and 24-methylchesta-7en-3-,6-ol are sub-components. Recently, ganodersterone has also been isolated.

2.5. Nucleosides and Nueleotides

Like other mushrooms, Reishi contains adenosine, 5'-GMP, 5'XMP, RNA, etc., components which contribute to umami. It has recently been found that nucleosides which contain adenosine and guanosine, found in the water/alcohol extract of Reishi, possess a platelet aggregation inhibition action(antithrotic activity).

2.6. Hypoglycemic Glycans and Proteoglycans

High hypoglycemic activity both in normal mice and in alloxan-induced hyperglycemic mice has been induced by two polysaccharide-protein complexes Ganoderan B and C, by their i.p. administration to male mice of Std:ddY strain. These complexes were obtained by adding ethanol to a hot water extract of Reishi and separating the precipitated polymer substances by column chromatography.

We have isolated, from Reishi fractions of water-soluble polysaccharides, a 3% ammonium-oxalate-soluble heteropolysaccharide, and a 5% NaOH-soluble peptidoglycan. These were further separated into several fractions by various chromatographic methods. Strong antitumor activity and hypoglycemic activity were found in certain fractions of the heteropolysaccharides.

No correlation was found between the antitumor activity of these active peptidoglycans and their antihyperglycemic activity or between the ratio composition of polysaccharides and proteins in the complex. Further research is needed to determine the source of these two activities.

2.7. Blood Pressure Stabilizing Components

Reishi has been assumed to have both hypotensive and hypertensive properties (homeostasis). A peptidoglycan (molecular weight, 100,000) having a mild hypotensive effect on Wister rats and SHR rats (congenitally hypertensive) has been isolated from a hot water extract of Reishi. According to one report, the blood pressure of about half the patients with hypertension was reduced when a Reishi extract was administered. It has been reported that a hypertension-related angiotensin-I-converting enzyme was inhibited by ganoderic acids (B, D, F, H, R, S, and Y), ganoderol A, and ganoderol A and B.

2.8. Antithrombotic Compounds (Platelet Aggregation Inhibitors)

Some active compounds have been isolated from mushrooms as platelet aggregation inhibitor. We have also isolated and identified adenosin, guanosin, and their derivatives as potent inhibitors from the 80% ethanol extract of Reishi. A novel substance showing a higher activity than those of the nucleotides was also obtained. Its structure has been identified as both epimers of 5'-deoxy-5'methylsulphinyl adenosine.

2.9. Lectins

Investigation of lectins from *G. lucidum* are now being carried out at our laboratory. We have isolated lectins from the fruiting body and mycelium of this fungus. This myceliumlectin is the first isolated from other than the fruiting body of higher fungi.

3. Antitumor Substances in REISHI

Mannentake (fruiting body and mycelium of *G. lucidum* fungus) contains various low molecular weight components. Free monosaccharides, sugar alcohols, oligosaccharides, amino acids, organic acids, steroids, lipids, terpenoids, coumarin,

tannin substances, etc., are among low molecular weight components extracted with various solvents of water/alcohol, water/acetone, water, etc.

3.1. Cytotoxic Terpenoids

It has been reported that some triterpenoids (ganoderic acid -R, -T, -U, -V, -W, -X, -Y, and -Z) isolated from cultured mycelia of *G. lucidum*, Mannentake showed a cytotoxicity-based carcinostatic effect on hepatoma cells in vitro.

3.2. Antitumor Polysaccharides

Among polymer components in Reishi, many polysaccharides and their protein complexes were extracted using hot water, ammonium oxalate solution, alkalisolution, dimethyl sulfoxide (DMSO) solution, etc., and separated by various chromatographic methods.

These polysaccharide fraction were used for the screening of hostmediated antitumor activities (BRM substances and immunotherapeutic agents) using Sarcoma 180/mice, i.p. or p.o. method. Strong antitumor activities were found in various hetero- β -D-glycans having a β -D-glucan chain as the active site, such as β -D-glucan, glucurono- β -D-glucan, arabinoxylo- β -D-glucan, xylo- β -D-glucan, manno- β -D-glucan and xylomanno- β -D-glucan, as well as their protein complexes. These polysaccharides, especially in Reishi, will be examined for their possible use as new antitumor agents.

In addition, polysaccharides having immunomodulated antitumor activities or anti-inflammatory functions are contained not only in water soluble β -D-glucans but also in hemicellulose (the so-called dietary fiber), which is water-insoluble. The polysaccharides present in hemicellulose can be extracted with alkali or DMSO in high yield.

In addition to these active polysaccharides, many other polysaccharides have been isolated or reported to exist, such as α -D-glucan (glycogen-like polysaccharide), fucogalactan, mannofueogalactan, fucoxylomannan and ylomannarabinogalactan. None of these showed antitumor activity.

3.3 Dietary Fibers

A high molecular component neither digested nor absorbed but excreted upon intake by human being is called the dietary fiber. Mushroom fungi contain dietary fibers belonging to β -glucan, chitinus substances, hetero-polysaccharides (pectinous substances, hemicellulose, polyuronides, etc.), and others, as much as 10-50% in the dried matter.

Since β -D-glucan and chitinous substance with carcinostatic activity are contained much in the dietary fiber of mushroom fungi, certain pharmacological effects can be expected, and further by physical actions they absorb hazardous matters as carcinogenic substances to prevent their absorption in the intestine and to hasten their excretion (laxative action), thus it seems to work effectively to prevent cancer of the colon and rectus.

3.4 Germanium Components

Crude drugs obtained from ginseng, a polyporaceae, treasured as Oriental medicinal have high germanium contents. The ability to concentrate Ge was confirmed in *G. lucidum* fungus. Correlation between the antitumor activity terferon-inducing activity and Ge contents is also of interest because Ge is said neutralize pain during the final stages of cancer.

4. Extracellular Polysaccharides of *G. lucidum*

Polysaccharides were produced extracellularly when *G. lucidum* mycelia were shaking cultured using a liquid medium containing monosaccharides or disaccharides as the carbon source.

Dried polysaccharides thus obtained were separated into water-insoluble (47%) insoluble (53%) fractions. The water-insoluble polysaccharide fraction contained a β -D-glucan having β -D-glucan-6-branches. When this glucan was administered to mice (10 mg/kg X 10, i.p.), it showed high antitumor activity, with the suppression ratio percentage of tumor proliferation being 92% and the complete regression ratio being 4/6. The watersoluble fraction contained a heteroglucan composed of glucose, mannose and galactose having no antitumor activity.

5. Clinical Study and Immunomodulating Effects of

***G. lucidum* Mushroom in AIDS and HIV Patients**

5.1. In Vitro Immunomodulating Effects of REISHI Extracts

Our preliminary in vitro studies indicated that certain extracts of Reishi may function as immuno restorer in mild to moderately immuno suppressed individuals. The effect may be mediated through changes T cell phenotypes or through enhancement of T cell function. Therefore, proper selection of the patients to be tested is essential to guarantee the favorable outcome of the clinical study.

5.2. Clinical Study of Micrinized REISHI Mushroom in Thai HIV Patients

A fine-powdered Reishi from a Japanese *G.lucidum* mushroom was administered to ten patients with advanced HIV infection in Thailand. All but nine patient had already taken antiretrovirals for two or more months before enrolling into the study and the CD4 count still remained under 200 cell/cu mm.

The same antiretroviral regimens were maintained throughout the 12-week study period. Patients were observed every 4 weeks for clinical, CD4 and HIV viral load changes. Results indicated that micronized Reishi mushroom was well tolerated. Nevertheless, the product did not show any appreciable benefits either the clinical symptoms, the CD4 counts or the HIV viral loads.

Therefore, it is desired to purify the active ingredients from the mushroom such as the terpenoids and polysaccharides including β -D-glucan and its protein complex before additional clinical test in HIV patients is warranted.

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Reishi