

Natural *Phellinus* Mushroom Story

Phellinus linteus, Natural Sang Hwang lingzhi has long been recognized as said in the old documents “If you can find a thousand years old lingzhi on an old Mulberry tree, you can save a dying person”. *Phellinus linteus* has been widely used in Japan, Korea and China. In 1993, Korea has succeeded in developing an anti-tumor medicine from *Phellinus linteus*.

In 1968, Dr. Ikegawa’s research team released their paper “Anti-tumor action of some basidiomycetes, especially *Phellinus linteus*” in a Japanese medicinal journal; *Phellinus linteus* became very popular among the researchers and scientists.

The efficacy of natural vs cultivated *Phellinus* mushrooms: “Artificially cultivated *Phellinus linteus* may be short on efficacy. Only natural ones are genuine *Phellinus linteus*.”

Phellinus linteus grows naturally in trunks of willow, paper mulberry and elm tree that is more than 100 years old, and shall grow for minimum 30 to 40 years to make a fruit body being used for medical application. In addition, the growth conditions such as humidity, temperature and lighting are very delicate, so that *Phellinus linteus* is very rare in the nature. That is the reason why, it is almost impossible to collect and take a fruit body in the nature.

Phellinus linteus is included in *Phellinus* genus of Hymenochaetaceae family of Aphylloporales order in mushroom class (Basidiomycetes) that mostly consists of fungi of medical use and edible fungi among molds. It is called Meshima in Japan (Danjo Island), Sanghwang in Korea.

Phellinus linteus is written as Sang-I in “Shin-Nong-Bon-Cho-Kyung” of China, Sang Hwang and Sang-Shin in Yak-Sung-Ron of Gyeon Ip-an in Tang era of China, Ho-son-an in Yu-Yang-Jap-Jo of Dan Seoung-sik, Sang-Hwang-Go in Chan-Yo-Gi-Bang, Sang-I and Sang Hwang in Bon-Cho-Gang-Mok of Lee Si-Jin in Ming Dynasty of China and Sang-I and Sang-Hwang in Dong-I-Bo-Gam of Her Jun. In addition, Big Dictionary of Chinese Medicine (Joong-Yak-Dae-Sajeon; Sanghai Science Publishing Company) has the terms such as Sang-Shin, Sang-I, Sang-Hwang-Go and Ho-Son-An.

In the tradition Chinese medicine, Sang Hwang (*Phellinus linteus*) was classified as Lingzhi or Reishi.

Sang Hwang lingzhi (*Phellinus linteus*) is the most highly rated herb category in terms of multiple benefit and absence of side effects. As a result from knowledge accumulated through 4000 years of human observation, it asserts that health can be maintained by sustaining the right balance with the body and the disease can be cured by restoring this balance through nutrition, including medicinal herbs, exercise and mental peace. In the other words, a disease is believed to be the tip of an iceberg, the result of the underlying imbalance of the body, which must be restored.

The 2000 years old medical book: Serg Nongs Herbal classic; considered today as the oldest book on oriental herbal medicine, classifies 365 species of roots of grass, woods, furs, animals and stones separates herbal medicines into 3 categories. The first category, called superior, includes herbs effective for multiple diseases and mostly responsible for maintaining and restoring the body balance. They have no unfavorable side effects. The second category, middle, comprises tonics and boosters and their consumption must not be prolonged. The third category, low, must be taken usually in small dosages and for specific ailments. This category includes some poisonous herbs.

Sang Hwang lingzhi (*Phellinus linteus*) ranked number one of the superior medicines, is the most exalted medicine in ancient times and has been used for perpetual youth and longevity. All observations show that Sang Hwang lingzhi (*Phellinus linteus*) has no side effects and can be consumed in high dosages and in parallel with other medications.

Sang Hwang lingzhi (*Phellinus linteus*) has been revered as herbal medicines for thousands of years in China and Japan.

Emperors of the great Chinese Dynasties and Japanese royalty drank tea and concoction of the Sang Hwang lingzhi (*Phellinus linteus*) for vitality and long life.

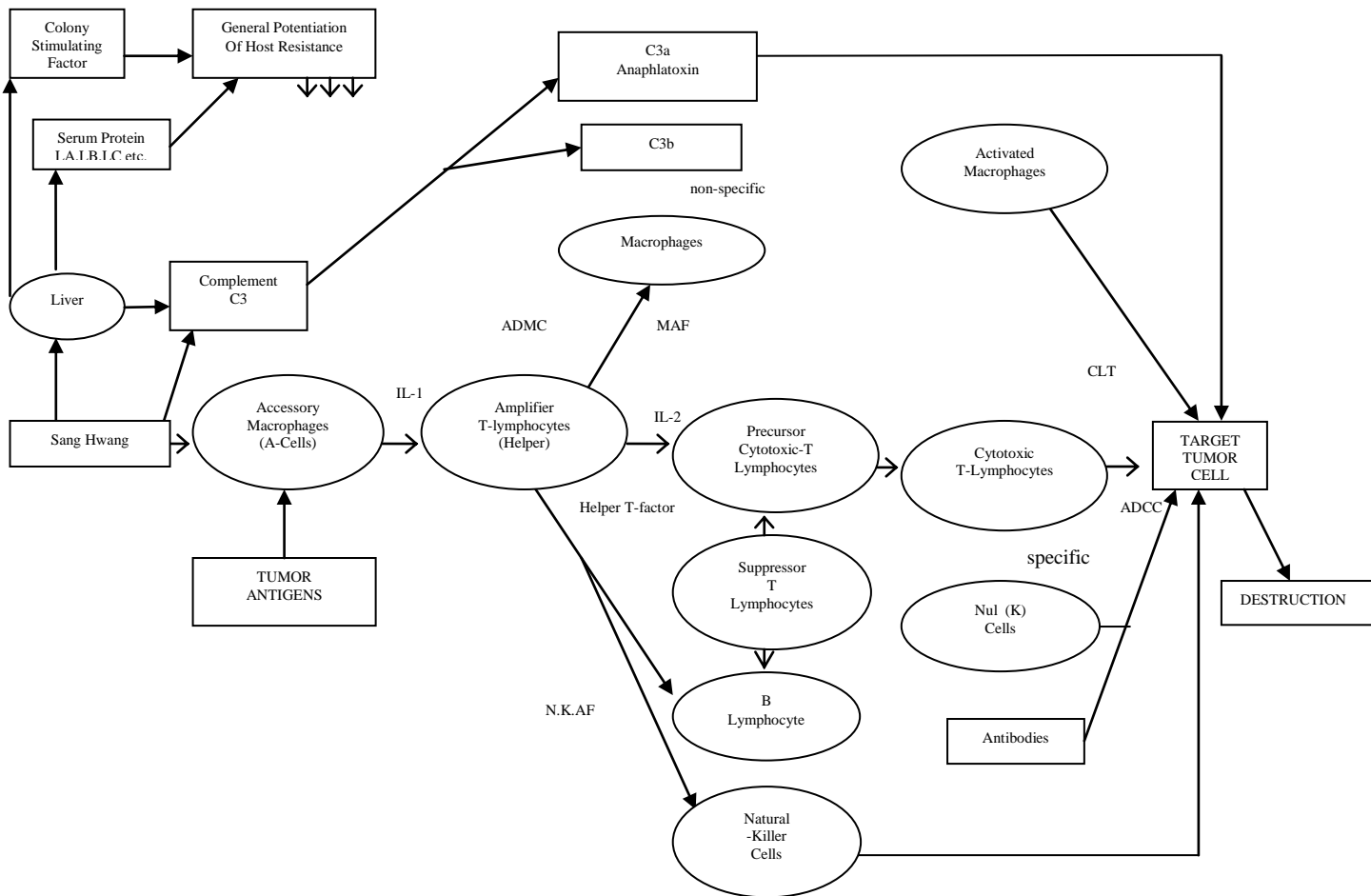
In Chinese History, the great Emperor Qin (Qin Chi Huang, 220 B.C.) once sent 3000 boys and girls to Japan's islands to search for a kind of lingzhi for the elixir of eternal youth. Sang Hwang lingzhi (*Phellinus linteus*) was believed to be the one. Why? If the *Ganoderma lucidum* is really the thousand years old Lingzhi, then; why Emperor Qin had to send 3000 people to Japan to look for this "Lingzhi for the elixir of eternal youth"? There had been so many *Ganoderma* in China, Why they needed to go to Japan?

In the 16th century pharmacopoeia: Pen Tsao Kang Mu, which contains hundreds of medicines the Chinese have used for thousand years, compiler Le Shih – Chen described the uses of Sang Hwang lingzhi. It positively affected the life energy or qi of the heart, repairing the chest area and benefiting those with a knotted and right chest. He wrote that it also increased intellectual capacity and banished forgetfulness. Taken over a long period of time, ability of the body would not cease and the years would be lengthened to those of the immortal fairies.

In China, the medical literature such as Shin-nou-nonzokyo (Imperial Medicine Handbook), Honzo-komoku (Medicine handbook), Chuusaku-daijitenn (Great dictionary of Chinese Medicines), Chuugoku-yakuyo-shinnkinn (Chinese medical Fungi), and Toyoigoku-daijitenn (Great Dictionary, 1967) describe the efficacy of palsy, gonorrhoea, and abdominal pain, and effective for helping urination disorder, stomach problems, hematuria, gripes, penis ache, prolapsus ani, melena, over-work, mense disorder, vitiated blood, lymphatic tumor, metrorrhagia, nosebleeds, facial and diarrhea. And in traditional Oriental applications, Sang Hwang lingzhi is also used to treat insomnia, gastric ulcers, neurasthenia, arthritis, nephritis, asthma, bronchitis, hypertension and poisoning. It is also being used in treating neuromuscular disorders, stress, induce tension, myasthenia gravis and muscular dystrophy – all with varying degrees of success. Presently, Sang Hwang lingzhi has various applications including lowering or raising blood pressure, stimulating liver actions, blood cleansing, and enhancement of the immune system and the lessening of nervous tension, and acting as an adaptogen in helping the body fight the effects of stress. It is also being recognized for its adjunct use as an immune system stimulator when cancer therapy is being used. The use of Sang Hwang lingzhi as a cancer treatment in the Orient is centuries old. In following the concept of qi tonics, Sang Hwang lingzhi is used to strengthen the body's resistance to outside forces.

When people continue to take the formulation medicine after boiling it for a long time, it works as a "miracle medicine", refreshing the human body, relieving ailments, and promoting longevity.

Natural sang Hwang can activate our immune system, increase the activation of Neutral killer cells about two times, T cell three times, B cell 129 times and macrophage 3 to 5 times.



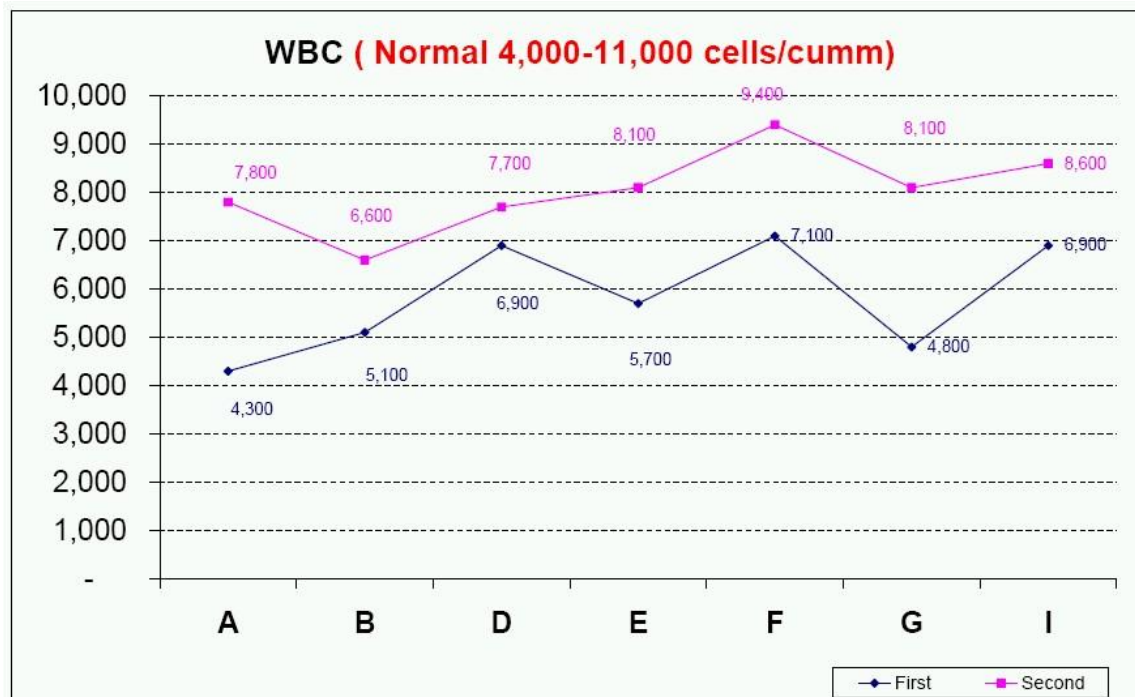
It is said that the immunity is the body protection army to make a body effectively prevent the intrusion from the outside (e.g. virus, bacteria and harmful chemical, etc.). Immunity treatment is to treat various diseases including cancer using the protection system inside a body. The immunity treatment is to exterminate disease as enhancing the immune capability that the function is weakened and increasing or decreasing the immune cells against the disease such as cancer and foreign substances that intrude the body.

Those cells to perform the immune functions are T cell. It suppresses the destruction and immune action of the cells that contain foreign substances such as cancer cell and helps the immune action. B cell produces substances to destroy foreign substances that intrude a body, macrophage that directly digests and decomposes foreign substances such as virus from the outside and neutral killer cell such as cancer cell that intrudes from the outside as activating the function of immunity cells that exist in a body.

When our immune systems work properly, our body will remain healthy. However, the immune systems in many people are weakened by stress, poor eating habits and pollution, like air pollution. These people can suffer from a number of diseases, such as frequent colds and Flu, allergies and asthma, chronic fatigue, fibromyalgia, candida yeast overgrowth, parasitic infections, psoriasis and eczema, rheumatoid arthritis, viral disorders, inflammatory bowel disease, hepatitis, cancer, diabetes, multiple sclerosis and lupus.

Natural Sang Hwang has been used traditionally for thousands of years and is now being validated by modern science for their remarkable healing potential. Natural Sang Hwang and their extracts has been the subject of hundreds of scientific studies which conclusively indicate their tremendous therapeutic value. According to these studies, natural Sang Hwang and their extracts show powerful immunomodulatory activity and enhance immune system function, anti-

tumor activity and inhibit the growth of existing tumors, help lower cholesterol and can prevent and treat cardiovascular disease.



(The above chart shows 10 people after taken the Sang Hwang extract 500mg per day for one month, Their White Blood Cells had been increased).

In the research of Natural *Phellinus linteus*, Dr Leo JLD Van Griensven and Dr. Huunb Savelkoul of Plant International, Wageningen University, the Netherlands stated that; *Phellinus linteus* induces the highest positive IL-10 response and a moderately positive IL-4 response. Also, IL-10 has been found to be a parameter for the effects of regulatory T cells. IL-10 is therefore assumed to balance the effects of Th1 and Th2 processes in the human body.

Natural *Phellinus linteus* extracts such as beta glucans that stimulate IL-10 production may then be assumed to be applicable in prevention resp. cure of diseases caused by dysregulation of the immune system. Those are allergies which are caused by excess Th2 processes, autoimmune diseases in which both Th2 and Th1 processes play an important pathogenic role and may be even in malignancies of the immune system i.e. T and B cell tumor (leukemias and lymphomas). Also those extracts could find application in repair of the immune system after chemotherapy for treatment of different cancers.

“To defend ourselves against invaders, be it foreign proteins, bacteria, viruses, parasites or even cancer cells, we, as higher vertebrates i.e. as humans, have a highly specialized and very active tissue in our body, i.e. our immune system in which a large number of white blood cells work together to inactivate the invaders.

The defensive effects of the immune system require a high rate of organization; we make not only antibodies that will recognize the foreigners in our body but also special killer cells that kill changed or foreign cells. Those two parts of our immune system must work closely together to be effective, and when the immunity is good, they are strongly regulated to do so. Normally that is the case: our immune system works as an effective surveillance to make us recover quickly from all kinds of infections, leaving cancer cells no chances to develop.

However, our immune system becomes weaker as we grow older; the surveillance becomes less effective and takes longer to recover. Some cancer cells get a chance to escape and will not be effectively killed anymore but can grow out to large tumors. In additions, the instructions to closely collaborate two parts of the immune system become more and more ignored.

Also the immune system may become sick or deregulated. This may already happen early in life, for example, it ignores instructions and making only little amounts of antibody against inhaled pollen in the air. We may get a pollen allergy with red eyes, running nose, sniffing and coughing as symptoms. Likely it may happen to food components with skin symptoms as a result. Actually, in the past 20 years, the incidence of various allergies has risen to 30 % among young children and teenagers.

On the other hand, the immune system may recognize normal proteins of our body as “foreign” and starts making antibodies against them and sends killer cells to remove the cells or organs containing those “foreign proteins”. That happens in case of autoimmune diseases such as rheumatoid arthritis, lupus, diabetes type 1, or chronic ulcerative colitis (Crohn’s disease), multiple sclerosis and many more. In all those cases, the functional cells of the body are harmed or killed, causing inflammation, swelling, pain, and disfunctioning.

A possible cure and prevention may be found in the regulation of the immune processes. Under normal healthy conditions, the immunity is regulated by a special type of small hormones that we call interleukins or cytokines. There are special interleukins that instruct the immune system to start or stop making antibodies and or kill cells. Under normal conditions, the amount of those hormones, their functions and their time of occurrence is finely balanced. They help us keep our body free of infection and cancer as well. The self balancing is done by a regulatory hormone and this seems to be one of the major players in the functioning of the immune system.

It has been found that some of the mushrooms used in the ancient Chinese medicine are effective because of their ability to induce the hormones guiding the immune system. Some mushroom preparations can be used as food supplements to stimulate inflammatory processes directed to fight “foreign proteins” by killer cells. Only the preparations of Phellinus linteus, Sang Hwang, have been found to fine balance the immune system, bringing it back to the healthy stage of young age. This may have very important implications. When used as a daily food supplement it may lead to strongly preventive activity of allergies or even to cure. It may help to stop inflammatory processes that make autoimmune disease dangerous and painful. It may even contribute to the prevention of spreading of some cancer cells in our body. “All this happens just because the immune system becomes balanced as in young age, and becomes as effective as in young age”, said Dr. Leo

The three major killers these days are cancer, cerebrosis and coronary diseases. The latter two have their etiology closely linked to the blood circulatory system. Related problems like stroke, heart block, arteriosclerosis, obesity etc. are all tied to problems in the blood circulation. One out of three persons died from one of the above ailments everyday. High incidences of these illnesses are closely linked to the adoption of the Asian dietary habits: heavy meat consumption, heavy carbohydrates and sodium consumption, lack of food fiber, change in basis food components when food is processed, frozen, and mixed with chemical additives etc. Consumption of these products provides fats and proteins in unbalanced proportions. This assimilation damages the blood vessels and oxygenation process to a marked degree that could affect the body’s metabolism. Yet, Sang Hwang lingzhi could correct this imbalance and strengthen the system to prevent further deterioration.

“In therapy of non-life threatening disease I would strongly suggest to find application in human allergies such as pollen and food allergy. Also in some cases of autoimmune disease i.e. in Psoriasis, Rheumatoid arthritis and in Crohn’s Disease, Phellinus extracts could be applied without important risk”, also said Dr. Leo.

Polysaccharide

It is known that various plants in nature has activating substances to treat the disease of human beings and even up to now, 75% of all human beings still use those plants to maintain the health. In the latter half of the 20th century, the concern on the bioactive substances that come from plants are increased due to the increase of chronic disease that it is very difficult to treat, the sudden increase of physical and psychogenic disease and serious side effects of compound medical goods. Those medical fluids using natural material are traditionally extracted by the hot-water extraction method and are mostly high molecular polysaccharides. However, all polysaccharides are not active and there are polysaccharides that are not active at all according to the stereostructure. There are polysaccharides with special activation and there are too many evaluation on polysaccharides because they are high molecular that contain various sugars.

The polysaccharide that holds the representative anti-tumor activity is protein polysaccharide that exists in basidiomycete (mushroom). In general, protein bound polysaccharide of basidiomycete has the anti-cancer effect as enhancing the immune function of a host as well as not having the poisonous effects on the normal cells differently from anti-cancer chemical therapies. So when it is combined with anti-cancer therapies, the treatment effect will be improved. Therefore, polysaccharide extracted from basidiomycete is a certain substance to adjust the biological reaction. Especially *Phellinus linteus* among those basidiomycetes has the strong anti-tumor effect and also has valuable factors such as (1,6)-heteroglycan and galctomannoglucan.

Polysaccharide from *Phellinus linteus* can be extracted by the methods such as hydrothermal extraction, ethanol extraction method, DEAE-cellulose chromatography, and gel-permeation chromatography. Polysaccharide comprise of 13.2% proteins and 82.5% saccharides, wherein 6.8% of the saccharides is uronic acid. MW of Polysaccharide is definitely 153 KD, determined by gel-permeation chromatography. It was confirmed from neutral glucose composition analysis that Polysaccharide consists of 7.0% of Ala, 3.7% of Xyl, 21.1% of glucose, 24.1% of Gal and 44.2% of Man, and from gas chromatography that uronic acid is a glucuronic acid. In the research of Natural *Phellinus linteus* made by Dr Leo JLD Van Griensven and Dr. Huunb Savelkoul of Plant International, Wageningen University, the Netherlands. Dr. Van stated that 19 kinds of amino acids have been found in Polysaccharide and Asp and Glu are major constituents. These amino acids are: Aspartate, Methionine, Cysteine, Threonine, Isoleucine, Serine, Luecin, Glutamate, Gluthathion, Tyrosine, Phenylalanine, Proline, Ornithine, Alanine, Lysine, Glycine, Histidine, Valine, and Arginine. But the cultivated *Phellinus linteus* has only Asparagine, Threonine, Serine, Glutamine, Alanine, Glycine, Valine, Isoleucine, Leucine and Lysine.

Saccharide parts and complete structure of Polysaccharide play an important role on physiological activity of Polysaccharide, but its protein parts are less significant. It has been found out that Polysaccharide is polymer comprising O-type linkage and that Polysaccharide is an immunoregulator for immunological enhancement. Polysaccharide improves entirely immune response. It has been confirmed by cell experiment and animal test that Polysaccharide enhances the immunological function of T-cells, macrophages, NK cells and B cells. Immunological enhancement of Polysaccharide is effective to suppression of cancer growth or metastasis. Polysaccharide controlled the growth and transference of animal's cancer cell, B16F10, and the growth of human solid cancer cells, NCI-H23.

Cancer treatment

Phellinus linteus (Sang Hwang Lingzhi) being been studied in Korea, Japanese and Chinese medicine for use as immuno-therapy agents and biological response modifiers (BRMs) for the possible treatment of cancer without side effects.

In recent years, the cultured mycelium product of *Phellinus linteus* has been developed as a medicinal in Korea as the result of a national project. It was approved as a medicine manufactured by Korean New Pharmaceutical Co., in 1997. This article summarizes the development and testing of medicine in Korea and the results of basic research and clinical data of Japan

Current anti-cancer treatment methods are surgical removal operation, radiation survey method and anti cancer chemical therapy treatment, but those methods have serious side effects and risks. Natural *Phellinus linteus* (Sang Hwang) is the body adjustment substance having an important role to exterminate cancer cells and foreign substances intruding from the outside. It also revitalizes the functions of various organs that are weakened due to the chemical therapy and the radiation therapy during the cancer treatment. In addition, it can activate the immune function, minimizes the side effects such as vomiting, weight reduction, mistrial and fallen hair, and enhances the immune function weakened by the surgical treatment such as the removal operation of cancer cells.

The human immune system is comprised of more than 130 subsets of white blood cells. About 15% of them are called Natural Killer (NK) cells; these provide the first line of defense for dealing with any form of invasion to the body. Each cell contains several small granules which act as ammunition. When an NK cell recognizes a cancer cell, for instance, it attaches itself to the cells outer membrane and injected these granules directly into organisms such as viruses and bacteria that enter the body. Through a process called phagocytosis, the NK cells “kiss” and engulf foreign agents and release toxic chemicals to destroy them.

In cancer patients, the NK cells and cancer cells constantly baffle one another. The NK cells attempt to destroy cancer cells while at the same time cancer cells try to neutralize NK cells before they can harm them. If the NK cells are weak or if there are not enough NK cells in the body, the cancer spreads throughout the body resulting in death. Several factors contribute to the weakening of the immune system: most notably, are stress, age, pollution, and smoking.

The research team of Dr. Chihara in Tokyo National Cancer Research Center in Japan found that hydrothermal extract of *Phellinus linteus* suppresses solid tumor sarcoma 180 a lot in 1976 (Protein & Nucleic acid Enzyme, pd155-157, Volume 59). As shown in the following table, the sarcoma 180 tumor is transplanted in a mouse. The hot water extract of 27 kinds of mushroom is injected in the right groin of mice to examine the suppression rate of tumor proliferation. As a result, it is found that hydrothermal extract of *Phellinus linteus* shows the predominant suppression effect as 96.7% compared to other mushrooms.

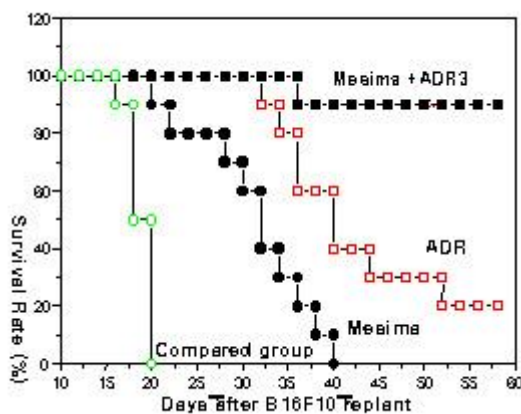
Table. Antitumor activity of hot-water extracts of mushrooms
(Solid Tumor Sarcoma 180 /mouse, ip method)

Name of Fungus	Inhibition rate (%)
Ganoderma applanatum	64.9
Coriolus versicolor	77.5
Coriolus hirsutus	65.0
Trametes gibbosa	49.2
Lenzites betulina	23.9
Daedaleopsis tricolor	70.2
Fomitopsis cytisina	44.2
Leucofomes ulmarius	44.8
Hirschioporus fuscoviolaceus	45.5
Coriolus pubescens	59.5
Favolus alveolarius	71.9
Fomes fomentarius	5.7
Fomitopsis pinicola	51.2
Ganoderma tsugae	77.8
Piptoporus betulinus	49.2
Trametes dickinsii	80.1
Phellinus h.	67.9
Phellinus igniarius	87.4
Phellinus linteus	96.7

Lentinus edodes	80.7
Flammulina velutipes	81.1
Pleurotus ostreatus	75.3
Pleurotus spodoleucus	72.3
Tricholoma matsutake	91.8
Pholiota nameko	86.5
Agaricus bisporus	2.7
Auricularia auricula-judae	42.6

Vaccinating sarcoma 180(a type of cancer cell) into the femur of these guinea pigs normally causes cancer to spread to the entire body within four to five weeks, resulting in the death of almost animals. The fungus extract is first administered 24 hrs, later when the cancer cells are firmly embedded in the animal's tissues, and the process continues for 10 consecutive days. The results are then recorded four to five weeks later. The experiment is repeated weeks after. The experiment is repeated in a group between five and ten guinea pigs, which each is given a different fungus extract. The mean values taken from these experiments are expressed as percentage. The anti cancer effect rate represents the percentage of guinea pigs which fully recovering from the cancer induced by an initial vaccination of sarcoma 180 and in whom a second vaccination of sarcoma 180 failed because the cancer cells could not be successfully embedded. From these results, it is deduced that the fungus extract (component primarily comprising a high molecular polysaccharide activates the immunity of normal biological tissue, so that even when a virus or other external factors enter the tissue, macrophage and interferon production within the tissue is vitalized to prevent the multiplication, metastasis and reoccurrence of cancer cells.

In general, protein bound polysaccharide of *Phellinus linteus* has the anti-cancer effect to enhance the immune function of a host, and does not have the poisonous effects on the normal cells; different from anti-cancer chemical therapies. But if we combine the Natural *Phellinus linteus* bound polysaccharide with anti-cancer therapies, the treatment effect will be much better than *Phellinus linteus* or chemotherapy alone.



In 2003, Dr. Tae Woong Kim, Dr. Jae Mo Sung, Dr. Ho Kyung Kim and Yoon Jung Ba of Kangwon National University, South Korea made a research on the “Antioxidative and anti-cancer effects of Natural *Phellinus igniarius* and Natural *Phellinus linteus* extracts”. The result has shown that the Natural *Phellinus igniarius* and Natural *Phellinus linteus* having a positive anti-oxidant and anti-cancer effect.

I. Materials and methods:

1. Preparation of samples; each sample is produced and designated as follows:

Abbreviation	Product name	Source
No 1	Water extract of <i>Phellinus igniarius</i>	Amazing Grace Health Products, Bangkok, Thailand
No 2	Water extract of <i>Phellinus linteus</i>	Amazing Grace Health Products, Bangkok, Thailand
No 3	Triterpenoids extracted from <i>Phellinus igniarius</i> plus Vitamin C.	Amazing Grace Health Products, Bangkok, Thailand
PI Ethanol	Ethanol extract of natural <i>Phellinus igniarius</i>	“Prepared as below”
PL Ethanol	Ethanol extract of natural <i>Phellinus linteus</i>	“Prepared as below”
PI water	Water extract of natural <i>Phellinus igniarius</i>	“Prepared as below”
PI water + vit.C	Water extract of natural <i>Phellinus igniarius</i> plus Vitamin C.	“Prepared as below”

2. Water extracts from *Phellinus igniarius* or *Phellinus linteus* (Products No. 1, No. 2 and No. 3); No. 1, 2, and 3 are products of Amazing Grace Health Products, Bangkok, Thailand.

3. Preparation of ethanol extracts of *Phellinus igniarius* and *Phellinus linteus*; Natural *Phellinus igniarius* or *Phellinus linteus* is dried at 50°C and crushed in a blender. The crude powder of natural *Phellinus igniarius* or *Phellinus linteus* is extracted with ethanol at 70°C for 3hrs (3 times). The extracts are evaporated at 60°C under pressure and, freeze dried for the antioxidative and anticancer tests. They are named as PI Ethanol (Ethanol extract of *Phellinus igniarius*) or PL Ethanol (Ethanol extract of *Phellinus linteus*), respectively.

4. Preparation of water extracts of *Phellinus igniarius*; Natural *Phellinus igniarius* is dried at 50°C and crushed in a blender. The crude powder of natural *Phellinus igniarius* is extracted with water at 100°C for 3hrs (2 times). The extract is centrifuged for 45 min. at 3500rpm. Supernatant was collected, and evaporated at 60°C under pressure, and freeze dried for the tests. They are named as PI water (Water extract of *Phellinus igniarius*) or PI water + vit. C (Ethanol extract of *Phellinus linteus* plus Vitamin C).

5. Antioxidative activities with DPPH radical scavenging method; 2ml of a 3.6×10^{-5} M ethanolic solution of DPPH are used. The decrease in the absorbance at 515nm is continuously recorded with a Shimadzu spectrometer for 16min at room temperature. The scavenging effect was plotted against the time, and the percentage of DPPH radical scavenging ability of the sample is calculated from the absorbance value at the end of 16min duration.

6. Cell culture; Cancer cells are Du145 (prostate adenocarcinoma) and SNU638 (stomach, ascite) cells. Normal cell (NIH3T3, fibroblast) is used. Adherent cultures are passaged at sub confluence after trypsinization. All the cell lines are maintained in RPMI1640 or DMEM medium supplemented with 10% heat inactivated fetal bovine serum, 100U/ml penicillin and 100ug/ml streptomycin. Cultures are maintained in humidified incubator at 37°C in the atmosphere of 5% CO₂ and 95% air until they reach confluence.

7. Anti-cancer assay; the above extracts of *Phellinus igniarius* or *Phellinus linteus* are dissolved in DMSO and diluted with medium. They are added to the cultures at the concentration of 0-200ug/ml each. For the growth inhibition assay, adherent (10,000cells) cancer cells are cultured in the 96-well culture plate in the presence or absence of the extracts of *Phellinus igniarius* or *Phellinus linteus*. Cell growth inhibition is observed by MTT colorimetric method. Briefly, 20ul of 5ug/ml MTT is added to each 96well. After 30 minutes incubation, 100ul of the culture medium is replaced with 100ul of isopropanol for the extraction of dye. All crystals are dissolved by repeated pipeting of the medium and measured by ELISA reader (570nm). Anti-cancer activity is calculated by the following formula: Anticancer ration (%) = $100 \times \text{OD}_{570} \text{ of test} / \text{OD}_{570} \text{ of control}$.

II. Results of experiments:

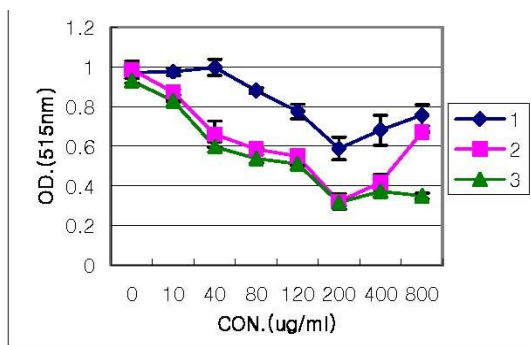


Fig 1. Antioxidative effects of *Phellinus igniarius*, *Phellinus linteus*, Triterpenoids.

- 1: Water extracted of *Phellinus igniarius*
- 2: Water extracted of *Phellinus linteus*
- 3: Triterpenoids from *Phellinus igniarius*

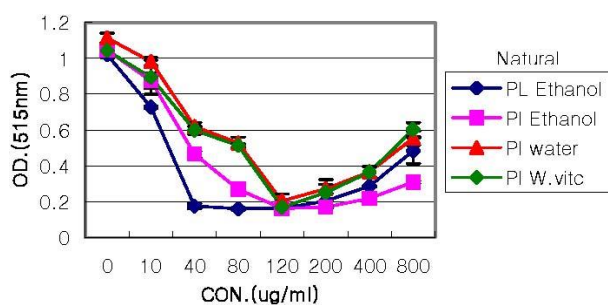


Fig 2. Antioxidative effects of ethanol and water extracts of *Phellinus igniarius*, *Phellinus linteus*.

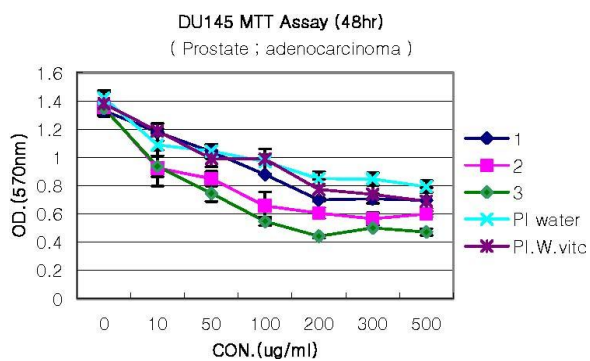


Fig 3. Anticancer effects of *Phellinus igniarius*, *Phellinus linteus*, Triterpenoids on Du145.

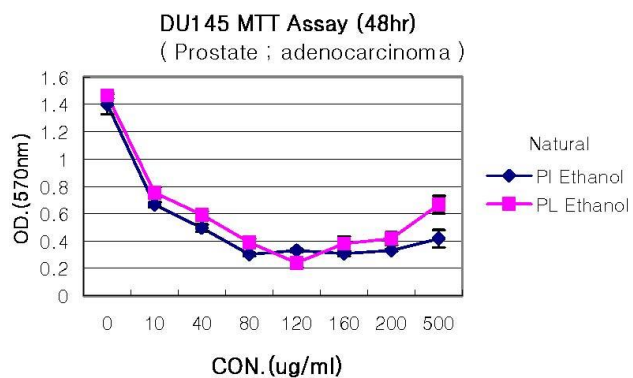


Fig 4. Anticancer effects of ethanol extract of *Phellinus igniarius*, *Phellinus linteus* on Du145.

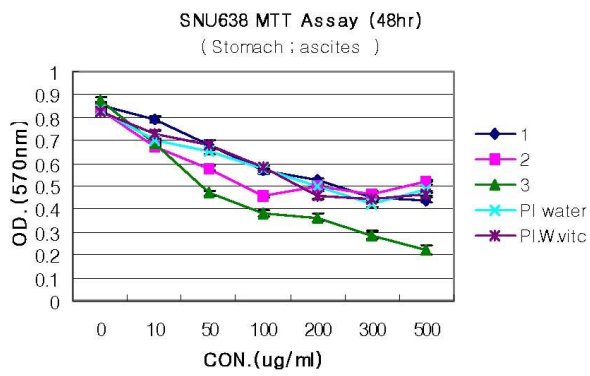


Fig 5. Anticancer effects of *Phellinus igniarius*, *Phellinus linteus*, Triterpenoids on SNU638.

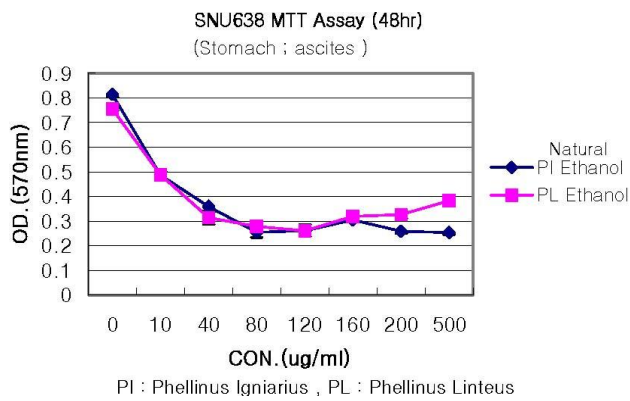


Fig 6. An anticancer effect of ethanol extracts of *Phellinus igniarius*, *Phellinus linteus* on SNU638.

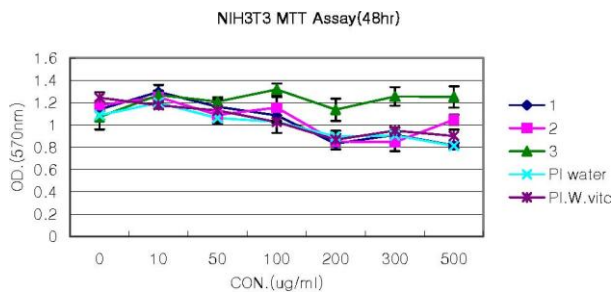


Fig 7. Effects of *Phellinus igniarius*, *Phellinus linteus*, Triterpenoids on NIH3T3.

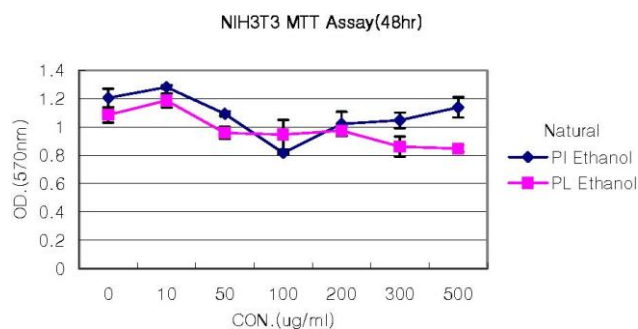


Fig 8. Effects of ethanol extract of *Phellinus igniarius*, *Phellinus linteus* on NIH3T3.

III Conclusions:

1. Antioxidative effects of *Phellinus igniarius*, *Phellinus linteus*, and Triterpenoids from *Phellinus linteus* (Fig. 1);

Sample 1): Water extract of *Phellinus igniarius*
 Sample 2): Water extract of *Phellinus linteus*
 Sample 3): Triterpenoids extracts from *Phellinus igniarius*

From the results (Fig. 1), antioxidative effects are as follows:

$$1 < 2 < 3$$

Therefore, Triterpenoids extracts from *Phellinus igniarius* (Item No.3) show the best antioxidative effects, followed by Water extract of *Phellinus linteus* (Item No.2), and Water extract of *Phellinus igniarius* (Item No.1).

2. Antioxidative effects of ethanol and water extract of *Phellinus igniarius*, *Phellinus linteus* (Fig. 2);

PL. E: Ethanol extract of natural *Phellinus linteus*
 PI. E: Ethanol extract of natural *Phellinus igniarius*
 PI. W: Water extract of natural *Phellinus igniarius*
 PI. W. vitC: Water extract of natural *Phellinus igniarius* plus Vitamin C.

From the results (Fig. 2), antioxidative effects are as follows:

$$PI.W = PI.W.vitC < PI.E \leq PL.E$$

Therefore, Ethanol extract of natural *Phellinus linteus* (PL. E) shows the best antioxidative effects, and followed by Ethanol extract of natural *Phellinus igniarius* (PI. E), and followed by Water extract of natural *Phellinus igniarius* plus Vitamin C. (PI. W. vitC) and Water extract of natural *Phellinus igniarius* (PI. W).

2. Anticancer effects of *Phellinus igniarius*, *Phellinus linteus*, Triterpenoids from *Phellinus linteus* on Du145. (Fig. 3);

1: Water extract of *Phellinus igniarius*
 2: Water extract of *Phellinus linteus*
 3: Triterpenoids extracts from *Phellinus igniarius*
 PI. W: Water extract of natural *Phellinus igniarius*
 PI. W. vitC: Water extract of natural *Phellinus igniarius* plus Vitamin C.

From the results (Fig. 3), anticancer effects were as follows:

$$PI.W \leq PI.W.vitC < 1 < 2 < 3$$

Five samples (stated above) are tested on DU 145(prostate adenocarcinoma) cancer cell to compare anticancer activities of extracts from *Phellinus spp...*

No. 3(Triterpenoids extracts from *Phellinus igniarius*) shows the best anticancer effect, and followed by No. 2(Water extract of *Phellinus linteus*), and followed by No. 1(Water extract of *Phellinus igniarius*).

Anticancer activity of PI. W. vitC (Water extract of natural *Phellinus igniarius* plus Vitamin C) or PI. W

(Water extract of natural *Phellinus igniarius*) is less than Samples, 1, 2, or 3 (Amazing Grace Health Products, and Bangkok, Thailand).

4. Anticancer effects of ethanol extracts of natural *Phellinus igniarius* and *Phellinus linteus* on Du145 (Fig. 4);

PI. E: Ethanol extract of natural *Phellinus igniarius*

PL. E: Ethanol extract of natural *Phellinus linteus*

From the results (Fig. 4), anticancer effects were as follows:

$$PL.E \leq PI.E$$

Ethanol extract of natural *Phellinus igniarius* or *Phellinus linteus* is compared anticancer effects on Du145 (prostate, adenocarcinoma). PI. E (Ethanol extract of natural *Phellinus igniarius*) and PL. E (Ethanol extract of natural *Phellinus linteus*) shows the similar anticancer activity, but, natural PI. E was slightly better than natural PL. E.

5. Anticancer effects of *Phellinus igniarius*, *Phellinus linteus*, Triterpenoids on SNU638 (Fig. 5);

Five samples (stated above) are tested on SNU638 (Stomach, ascite) cancer cell to compare anticancer activities of extracts from *Phellinus spp.* Results (Fig. 5) from SNU638 (Stomach, ascite) showed very similar anticancer results from Du145 cancer cell (Fig. 3).

From the results (Fig. 5), anticancer effects are as follows:

$$PI.W \leq PI.W.vitC \approx 1 < 2 < 3$$

No. 3 shows the best anticancer effects, followed by No. 2, and by No. 1 respectively. Anticancer activities of Samples, 2 and 3 (Amazing Grace Health Products, Bangkok, Thailand) are much better than natural PI. W or PI. W. vitC.

6. Anticancer effects of ethanol extracts of *Phellinus igniarius*, *Phellinus linteus* on SNU638 (Fig. 6);

PI. E: Ethanol extract of natural *Phellinus igniarius*

PL. E: Ethanol extract of natural *Phellinus linteus*

From the results (Fig. 6), effects are as follows:

$$PL.E \leq PI.E$$

Ethanol extract of natural *Phellinus igniarius* or *Phellinus linteus* was compared anticancer effects on SNU638 (Stomach, ascite). PI. E (Ethanol extract of natural *Phellinus igniarius*) and PL. E (Ethanol extract of natural *Phellinus linteus*) show the similar anticancer activity, but, natural PI. E is slightly better than natural PL. E as shown on Fig. 6.

7. Effects of *Phellinus igniarius*, *Phellinus linteus*, Triterpenoids on NIH3T3 (Fig. 7);

To test the cytotoxicity of *Phellinus spp.*, normal cell (NIH3T3, Fibroblast cell) is used for the test of cytotoxicities of *Phellinus igniarius*, *Phellinus linteus*, and Triterpenoids.

But, all extracts do not show any significant cytotoxicity on normal cell line (NIH3T3, fibroblast) as shown on Fig. 7.

8. Effects of ethanol extracts of *Phellinus igniarius* and *Phellinus linteus* on NIH3T3. (Fig. 8); Fig.

8 shows that ethanol extracts of *Phellinus igniarius* and *Phellinus linteus* do not show any significant cytotoxicity on normal cell line (NIH3T3, fibroblast).

From the results, it is concluded that *Phellinus spp.* is effective to reduce cancer cells (DU145 and SNU638), Furthermore, *Phellinus spp.* does not show any cytotoxicity on normal cell (NIH3T3, fibroblast). And, products No. 1, 2, and 3 (Amazing Grace Health Products, Bangkok, Thailand) are very effective to reduce cancer cells such as DU145 and SNU638,

Intake dosages of *Phellinus linteus*

Phellinus linteus is a well known medicinal mushroom that contains immunomodulatory polysaccharides. Polysaccharides isolated from *Phellinus linteus* have been used in several scientific studies and have been found to be effective in vertebrate systems against tumor cells in vitro and against solid tumors in vivo. Their effect is thought to be intermediated by the induction of specific cytokines that are able to enforce cellular and humeral immunity.

In 1996 Kim et. al. had found that polysaccharide extracts from *P. linteus* enhancing in vitro and in vivo immune function of mice. In their animal experiments they had applied **100 mg** extract per kg body weight and in vitro they had used up to **1 mg per ml** culture fluid.

In a study (Han et. al. 1999) on the immunotherapeutic activity of *P. linteus* polysaccharides against tumor growth and metastasis it was found that *P. linteus* inhibited tumor growth in nude mice with implanted solid tumors (melanomas), prolonging their survival rate and reduced the frequency of metastasis. In those experiments, the mice were given daily **intraperitoneal** injections at **100 mg** polysaccharide per kg body weight for 12 consecutive days.

Ikekawa (2000) has shown that polysaccharides isolated from *P. linteus* causing complete regression of the transplantable Sarcoma 180 in mice when those mice were **orally** given **200mg** *P. linteus* polysaccharides per kg body weight per day for a period of 10 days.

In a study on the prevention and treatment of collagen induced arthritis in mice Kim et al (2003) administered polysaccharides they had isolated from *P. linteus* identically to the authors above. They applied intermittent **oral** administration of **200 mg** per kg body weight and found a decrease in arthritic symptoms. They concluded that proteoglycan (read polysaccharide) from *P. linteus* is good for preventing as well as remedying of collagen induced arthritis.

Ajith & Janardhanan (2003) have described the cytotoxic and antitumor activities of a close relative of *P. linteus*, i.e. *P. rimosus*. They used oral administration of polysaccharides in mice at a dose of **50 mg** per kg body weight per day for 10 consecutive days and found a 57% (transplantable) tumor growth inhibition. Interestingly, their polysaccharides were non cytotoxic for their tumor cells in vitro, not even at **1 mg per ml** culture fluid.

“In my opinion, *P. linteus* polysaccharides extracts could be used at doses of up to 200 mg per kg body weight per day for reasons of effectiveness”, Dr. Leo said.

Cytotoxicity

Cho et al. (2002) used *P.linteus* Polysaccharides in a model study on mechanisms of inflammation (= cellular immunity) and observed that in rat liver epithelial cells *P.linteus* extracts were non toxic at even **10 mg per ml** culture fluid for 24 hours.

It should be noted that rat liver epithelial cells are an important model cell system because those

Cells like skin fibroblasts are not yet transformed and therefore reflect natural cells better than tumor lines.

As far as acute oral toxicity is concerned Han et al. (2001) have tested toxicity of *Phellinus linteus* extracts in rate in vivo. They found that single doses of extract of up to **2000 mg** per kg body weight did not affect the health of the rats; body weight remained normal as did food and water intake. No negative findings were reported upon autopsy of the rats that had received up to 2000 mg per kg. Even at 5000 mg per kg no negative clinical signs were observed.

“In my opinion, *P. linteus* polysaccharide extracts could be safely used in humans at doses of up to 200 mg per kg body weight per day”. Dr. Leo

Also, another research made by Yong Suk HAN, Soon Young PARK¹, Byung Ki CHOI² and Se Young CHOUNG in Kyung Hee University and Dongduck University, Korea.

“Acute Oral Toxicity Studies of Extract of *Phellinus linteus*”:

The current study was performed to determine the acute oral toxicity of a crude extract of *Phellinus linteus*, in SD rats. 5 rats of each sex were orally treated with a single dose of extract of *Phellinus linteus* mushroom at doses of 0, 500, 1,000, 2,000 mg/kg, respectively. After the treatment, clinical signs and body weight change, the food and water consumption were observed for 14 days. All animals survived during the study and did not show any clinical signs. Body weight gain showed no significant difference between the control and treated rats. However, body weight gain delayed in high dose group (2,000 mg/kg) on day 1~3 after administration. Another 5 rats of each sex were orally treated with a single dose of extract of *Phellinus linteus* mushroom at dosages 4,000, 5,000 mg/kg respectively, but all animals survived during the study and did not show any clinical signs. It is suggested that LD50 of extract of *Phellinus linteus* mushroom by oral administration was estimated to be over 5,000 mg/kg in both sexes of rats.

Natural *Phellinus linteus* and Diabetes

On 26 October, 2004. *Phellinus linteus* has registered its patent: “Compositions containing polysaccharides from *Phellinus linteus* and methods for treating diabetes mellitus using same” Patent: 6,809.084 in United States

Diabetes research has been directed toward prevention and cure of insulin-dependent diabetes mellitus. Studies on prevention of insulinitis and treatment of diabetes have mainly utilized the experimental model of diseases in laboratory animals such as NOD mice, and most therapeutic strategies for treatment of diabetes mellitus are directed to suppression and regulation of autoimmune response in order to prevent beta-cell destruction. Autoimmune disease, insulin-dependent diabetes mellitus results from increasing abnormalities of cellular immunity.

Various immunotherapies for preventing destruction of pancreatic beta-cells have been attempted. Neonatal thymectomy is the method of suppressing the outbreak of diabetes mellitus in NOD mice by killing T lymphocytes. Also, it has been known that depletion of macrophage of T cells using antibodies to T cells-dependent antigens represses diabetes. Some reports disclose that diabetes mellitus can be prevented by controlling production and reaction of free radical.

To date, researches on immunosuppressive therapy are continued. However, the treatment of diabetes mellitus utilizing glucocorticoids and cyclophosphamide has proved to be largely unsuccessful. Although studies on the use of cyclosporin A, rapamycin, and FK506 in diabetes appear to be encouraging, generalized immunosuppression involves potential complications including infections and drug-induced kidney and liver damage. Furthermore, curing with long-term administration can occasionally induce cancer. Studies on immunosuppressive therapy for diabetes mellitus is progressing, and the suppression of diabetes of NOD mice using cytokines such as IL-4 and IL-10 has been reported. Also, such immuno-regulatory agents as OK-432, LZ-8, BCG and CFA have been reported to control diabetes mellitus of NOD mice, but their mechanism destruction of pancreatic beta-cells, resulting in prevention and cure of diabetes mellitus.

Doses of the inventive PL are 100 mg/kg/day every other day with the intraperitoneal injection. The dose level, route, and schedule of administration however may vary, depending on the condition of the subject. PL suppressed completely crisis of diabetes mellitus of NOD mice. Prevention and cure of diabetes mellitus by PL is examined by the test, in which the urine or blood glucose level had been compared to that of control and significant drop was observed. PL inhibited lymphocyte infiltration into pancreatic ducts, to suppress insulinitis.

The activation of T lymphocytes and macrophages plays an important role in autoimmune disease system. IL-2 and IFN-gamma, which are cytokine from Th1 cells, enhance autoimmune responses. IL-2 promotes a destruction of beta cells by activating cytotoxic T cells (CTLs). IFN-gamma can activate macrophages, and the activated macrophages amplify the antigen presenting function to increase autoimmune responses. Simultaneously, free radicals such as activated oxygen may be increased, resulting in a destruction of beta cells. Increase of IL-10 or IL-4 cytokine expression by cytokines from Th2 leads to inhibition of function of Th1 cell and consequently decline in autoimmune responses. IL-6 and IL-12 are cytokines expressed by macrophages. Particularly, IL-12, which stimulates Th1 cells to enhance the production of IL-2, performs an important function in inducing autoimmune diseases such as diabetes mellitus. PL inhibits the expression of such cytokines as IL-2, IFN-gamma, IL-10, IL-6 or IL-12, which plays an important part in the occurrence of diabetes mellitus. Although the increment of secretion of IL-4 from Th2 is thought to be a significant reason of the decline of Th1 cell function, nothing is supposed to do with the decrease in the expression of IL-10. PL's regulating function works as a major factor of suppressing diabetes mellitus.

The important feature of this invention is that PL has a curing effect on diabetes mellitus without any negative effects. Since NOD mice have received PL, they have showed no weight gaining, nor any signs of being killed. PL is expected to have no side effects.

Insulin-dependent diabetes mellitus can be induced by streptozotocin. Streptozotocin destroys selectively pancreatic beta cells, and diabetes mellitus induced by streptozotocin is similar with diabetes naturally occurring in MOD mice. NOD mice mediate the autoimmune responses to break the beta cells, whereas streptozotocin does not do that. PL could not suppress the destruction of beta cells occurring by streptozotocin. This result shows that PL is a substance which regulates selectively the immune responses to suppress the outbreak of diabetes mellitus.

Research of *Phellinus Linteus* in Japan and Korea

- 1) Clarified the antitumor activity of Basidiomycetes (especially *Phellinus linteus*) Polysaccharide for the first time in 1994 (*National Cancer Research Center, T. Ikekawa, G. Chihara et al.; Tokyo University Medicine Department, S. Shibata et al. in 1968*).
- 2) Administered hot-water extract of the cultured mycelium of meshimakubo to 24 patients suffering from stomach, colonic lung, and uterine cancer. (*Of these, 12 improved, 6 cases were*

unconfirmed, and 6 worsened. Saijo Central Hospital (Hiroshima) Immunophysics Department, S. Yamana (1980 Japan)).

- 3) Culturing method of spawn body with anticancer activity. (S. Yamana: Japan Open Patent Gazette (A) 1991-175974).
- 4) Sumitomo Memorial Hospital (Aichi Pref.), Y. Naito orally administrated Meshima to a terminal lung cancer male patient of 50 years old, 2-3 g. per day by dividing in three portions. The tumor marker dropped after 1 month from 1.7 to 0.7 and then to normal. In a mammary cancer female patient in her 30s, who had been suffering from severe pain due to a tumor the size of a table tennis ball at her left breast, the tumor disappeared 2 months after she started to take Meshima.
- 5) Korea started to sell Meshima, the mycelium product of *Phellinus linteus* to Japan in 1998. Meshima Japan Co., which had contracted with Korea New Pharmaceutical Co., became the manufacturer and wholesaler in Japan in 1999.
- 6) Administer Meshima in the following cases as substitute medical treatment against cancer: (*Ebisu garden press clinic (Tokyo), S. Takagi, 1999*).
 1. It is administered for the prevention of recurrence, after a successful operation, with complete removal of the tumor, as judged by supersonic inspection or X-ray inspection.
 2. It is administered if the entire tumor could not be removed during the operation, or an operation is impossible. Its purposes are to suppress the growth of cancer, improve quality of life, or prolong of life.
 3. It is continuously administered when an operation is impossible because of the age of the patient or religious beliefs.
- 7) It is confirmed by animal tests with mice that the mycelium extract (Meshima) of *Phellinus linteus* can suppress cancer not only by injection but also by oral administration. (*Kanazawa University, Medicine Department, T. Ohta, 1999*).
- 8) A cultured mycelium extract, Meshima Gold has been developed by Applied Microbiology Laboratory Obiken Co., Ltd., in 1999.
- 9) After obtaining some information from the study on Meshimakobu in Japan, the national project among Korean scientists and pharmaceutical companies proceeded with research and development. Pharmaceutical production based on the hot-water extract of the cultured mycelium was successful, and Korean New Pharmaceutical Co., was approved to produce the medicine Meshima. An immunity-enhancing agent, Meshima capsules, was awarded the so called Korean Nobel Prize, the Chazan Prize, in 1998.
- 10) The hot-water extract of meshimakobu, increased the macrophage activity of a mouse fivefold, and delayed type hypersensitivity reaction of T-lymphocytes increased threefold. (*Seoul University, College of Pharmacy, B.K. Kim et al*).
- 11) The effects of Meshima are as described. Spleen B cells and macrophages are strengthened. The antibody reaction of the mouse is strengthened 129 fold. The ability to form lymphocytes is strengthened 3.3 fold. An increase in the number of lymphocytes in the abdominal cavity is induced, and cancer cell killing ability is strengthened. The cancer preventive effect by administration prior to transplantation of cancer cells is also increased. (*Chung Nam University, College of Pharmacy, K.S. Chung et al*).
- 12) Antibody generation ability and immune enhancing effects were each strengthened fivefold. A new saccharide was developed from the culture solution of meshimakobu

mycelium. The life of a cancer-carrying mouse was prolonged by 1.8 times by the administration of meshimakobu. (*National Life Science Institute, I-D. You. H.M. Kim et al*).

- 13) The safety of Ld > 1500 mg/kg has been confirmed. The life of a mouse that is infected with abdominal dropsy by transplantation of mouse sarcoma 180 is prolonged by 5-6 days. Anticancer activity is expressed by ordinary immunity mechanisms as a result of the two-to threefold enhancement of natural killer (NK) cell activity. (*Kyung-Hee University, Tong-Eut Medical Institute, N-D. Hong et al*)
- 14) The commercialization of hot-water extract of cultured mycelium of meshimakobu has succeeded. The trade name Meshima has appeared in the market after the approval as a medicine. (*Korean New Pharmaceutical Co. and Natural Life Science Institute, 1993*)
- 15) After the medicine was applied to cancer patients: 9 cases of stomach cancer, 2 cases of liver cancer, 2 cases of unidentified cancer, and 6 cases of compound cancer, the side effects were not apparent and immunity (helper T cell) of patients improved. (*Chung-Nam University, S.-Y. Kim et al*).
- 16) The extract high molecular weight substances is applied from the fruiting body of cultured mycelium and cultured liquid of the fruiting body then measured their immune activity. The results are 65.77%, 41.95% and 21.87% respectively. (*Takeu Engineering University, Ch.-F Song*)