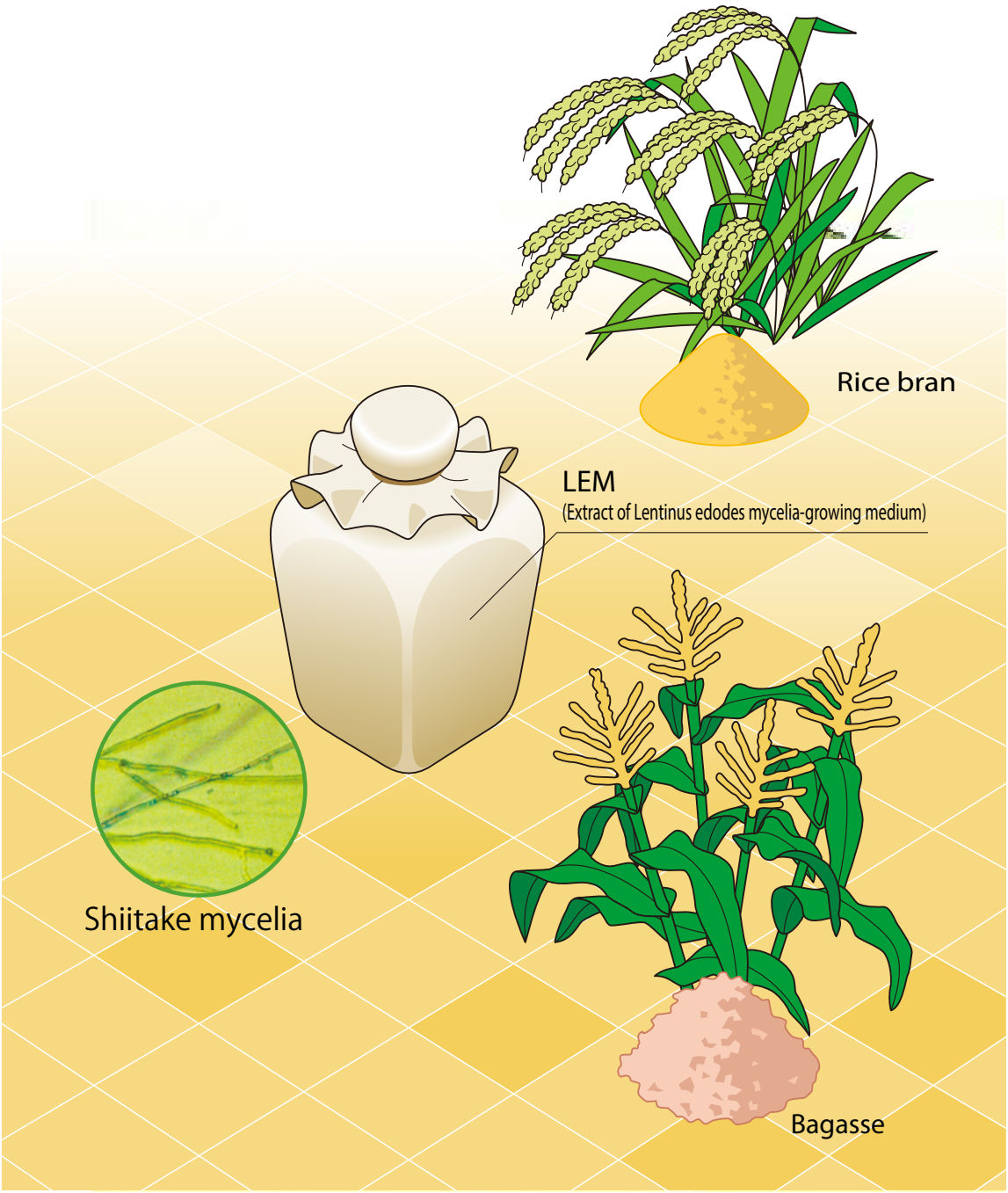
The LEM Guidebook

The Mysterious Power Derived from Shiitake Mycelia



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Introduction

The Curious Powers of LEM

1. The Fascinating of Shiitake Mushrooms

LEM is a unique fermented product made by using the powerful natural ability of shiitake mushroom mycelia to break down organic matter. Shiitake mushrooms are commonly grown and enjoyed as food in Japan, China, and South Korea, and can also be found in Southeast Asia and New Zealand. In Japan, shiitake cultivation began during the Edo period. Back then, growers would gently damage the surface of broadleaf trees like beech and oak, patiently waiting for mushroom spores to settle and sprout naturally. After that, artificial spore inoculation techniques were developed, enabling more efficient shiitake cultivation. Today, shiitake mushrooms are grown year-round.

Shiitake mushrooms are the most widely grown and consumed mushroom in Japan. Although low in calories, they contain a variety of essential nutrients, especially vitamin D, vitamins B1 and B2, dietary fiber, and potassium. These nutrients have been linked to several health benefits, such as lowering blood pressure and cholesterol, reducing the risk of blood clots, boosting antiviral defenses, and even supporting the body's fight against tumors.

Around 40 years ago, scientists discovered that certain polysaccharides found in mushrooms such as shiitake, enoki, and kawaratake (turkey tail) can stimulate immune responses that help fight tumors. In 1985, lentinan, a β-glucan extracted from shiitake, was approved as an anti-tumor agent, which sparked growing interest in mushroom-based immunotherapy.



2. The Expansion of Complementary and Alternative Medicine and the Role of LEM

Interest in complementary and alternative medicine (CAM) is steadily growing. The human body is incredibly complex, and our health is affected by many factors including stress and the surrounding environment. Even with cutting-edge medical treatments, some illnesses are still difficult to cure.

The rise of the internet has made it easier than ever to access health and medical information. While this can be empowering, it has also led to new challenges. Patients and their families often struggle to sort through the overwhelming volume of information, which can create confusion and anxiety.

In response to this growing need for guidance, the Japanese Society for Complementary and Alternative Medicine published a “Guidebook for Complementary and Alternative Medicine in Cancer Treatment” in April 2006, with support from Japan’s Ministry of Health, Labour and Welfare. This guidebook offers practical advice on how to approach complementary treatments with clarity and caution.

Japan’s CAM landscape is evolving, but efforts have lagged behind those of Western countries. For example, Japanese consumers tend to rely heavily on health foods and supplements, while massage and acupuncture are more common in the West. Unfortunately, many supplements in Japan are used without sufficient scientific validation, raising concerns about their reliability and safety.

LEM has been the subject of extensive basic and clinical research over the years, leading to discoveries of biological mechanisms that support the elimination of bacteria, viruses, and cancers. Research is ongoing, and there is growing interest in its potential roles within the nervous system and other physiological areas, which may offer promising paths for future insights.



Chapter 1: How LEM Is Made

1. What Is LEM (Extract of *Lentinus edodes* mycelia-growing medium)?

Shiitake mushrooms are a beloved part of Japanese cuisine and are traditionally grown on special wooden logs. While the shiitake mushroom appears to be the main body, its true essence is the mycelium that lies deep within the log, the network of thread-like cells hidden inside the log. The shiitake mycelia use its own enzymes to break down wood, turning it into nutrients for growth. When conditions are just right, it produces the fruiting body we recognize as a mushroom, which is simply a tool for reproduction.

The principle behind LEM production was discovered by chance during a mycelial growth test accident, and the method was later patented in 1971. It's a brown, water-soluble substance extracted from a medium made of bagasse (the fibrous by-product of sugarcane) and rice bran, used to grow shiitake mycelium. The extraction takes place before the mushrooms start forming. The name "LEM" combines the initials of the shiitake's scientific name, *Lentinus edodes*, and the word "Medium."



Photo 1. Cultured solid medium

The late Nobuhiko Kanno, PhD, Professor Emeritus at Toyama Medical and Pharmaceutical University and a pioneering researcher of LEM, is credited with naming it "Extract of Lentinus edodes mycelia-growing medium."

2. LEM Manufacturing Process

The carefully designed production process of LEM is described in detail. Its primary raw material is bagasse, the fibrous residue remaining after sugar is extracted from sugarcane, which is blended with rice bran to create a culture medium. This medium is placed into bags and then heat-sterilized. Pre-cultured shiitake mycelium is injected into the sterilized bags, which are stored in a cool, dark environment where the mycelium slowly grows.

The bagasse used is sourced through production contracts with domestic sugar manufacturers in Japan. Once the mycelium fully colonizes the medium (about four months later), it is transferred to extraction tanks and heated. Enzymes facilitate the digestion of the mycelium and the medium's components, releasing active substances into the extract. After adjusting the extract's concentration, LEM is complete. In some cases, LEM is further processed into a solid form.



Photo 2. Bagasse

Chapter 2: Components and Function of LEM

1. Two Key Active Ingredients:

LEM's biological functionality centers around two key active compounds: polysaccharides and water-soluble lignin, which are uniquely generated through enzymatic processes in shiitake mushroom mycelia. During fermentation, these enzymes act on the high-molecular-weight, non-water-soluble components of bagasse and rice bran, converting them into water-soluble polysaccharides and lignin with significantly lower molecular weights.

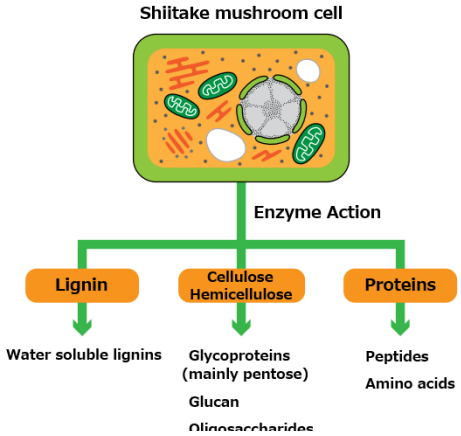
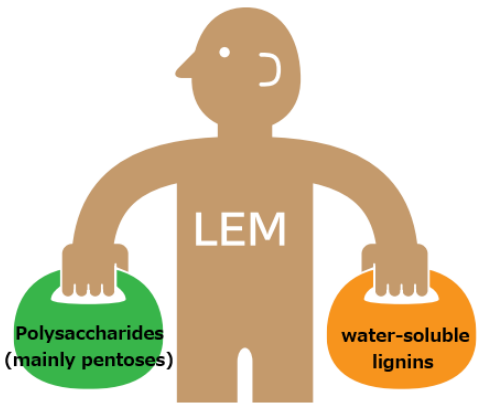


Fig.1. Production of active ingredients by shiitake mycelia

2. Polysaccharides

The polysaccharides in LEM are distinctive, as they are primarily composed of pentoses such as arabinose and xylose, with most having molecular weights below 200,000. This relatively small size may allow for easier absorption. The β-glucans in mushrooms differ significantly from LEM, as they are composed exclusively of glucose (a hexose) and can have molecular weights in the millions. LEM's polysaccharides offer a unique composition and size that could contribute to distinctive functional benefits



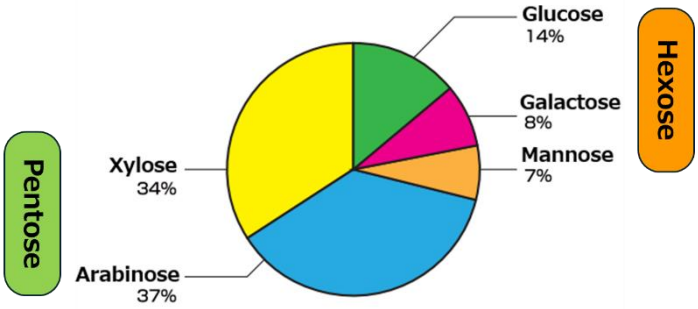


Fig.2. Monosaccharides Composed of LEM

Pentoses: Monosaccharides containing five carbon atoms (e.g., xylose, arabinose)
Hexoses: Monosaccharides with six carbon atoms (e.g., glucose, galactose, mannose)

3. Water-Soluble Lignins

Lignin is one of the key substances found in plant cell walls, working alongside cellulose and hemicellulose to protect the plant's structural integrity. Under normal conditions, lignin is a very large molecule with a molecular weight that can reach into the millions, making it water-insoluble and highly resistant to breakdown.

Interestingly, shiitake mushroom mycelia contain enzymes capable of breaking down lignin. When they act on bagasse (a fibrous plant residue), they appear to transform the original lignin into smaller, water-soluble molecules. This process likely results in the formation of new water-soluble lignin compounds.

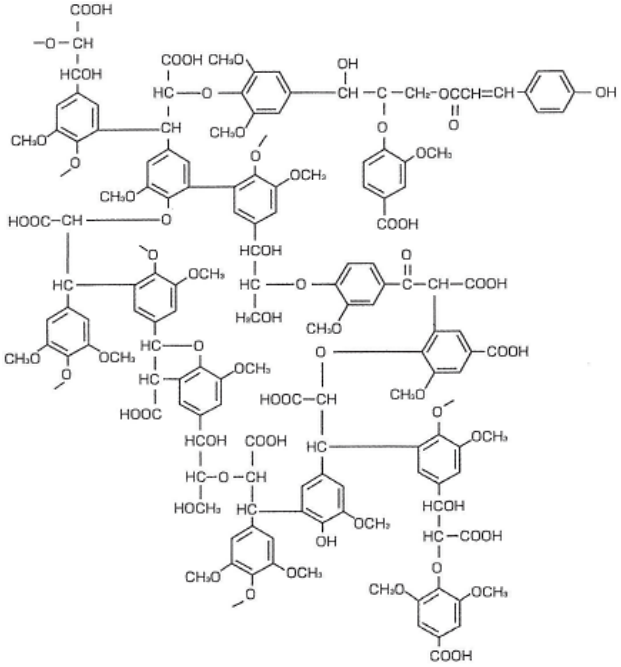


Fig.3. Example of A Putative Structure of a Water-Soluble Lignins

Table 1. Comparison of polysaccharides and water-soluble lignins in LEM

	Immune-Enhancing Activity	Direct Effect
Polysaccharides (mainly pentoses)	1. Activation of macrophages 2. Enhancement of killer T cell cytotoxic activity 3. Enhancement of antibody production	• Not observed
Water-soluble lignins	1. Activation of macrophages 2. Enhancement of NK cell cytotoxic activity	• Antiviral Activity • Antitumor Activity • Antiallergic Activity • Reactive oxygen species Scavenging Activity

Chapter 3: How LEM Helps Your Body Defend Itself (Immunostimulatory Action)

1. How LEM Activates the Immune System and Clears Out Invaders

Every day, our bodies encounter invisible intruders such as bacteria, viruses, and other foreign substances through ordinary actions like breathing and eating. Fortunately, the immune system functions as a highly specialized defense team. When it detects something unfamiliar, cells including macrophages and helper T cells respond rapidly. This triggers the production of antibodies, cytotoxic T lymphocytes (also known as killer T cells), and other immune components that work together to eliminate foreign substances (Fig. 4).

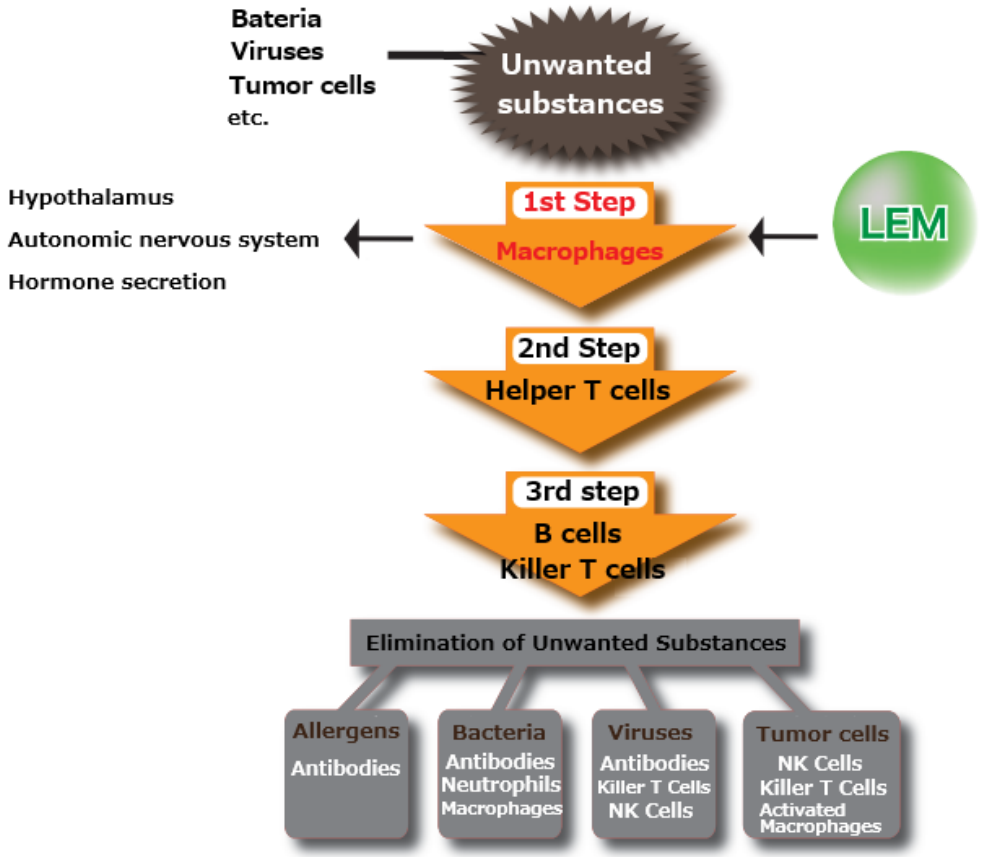


Fig.4. Overview of Immune Activation and Clearance Process Unwanted Substances

Recent studies show that the immune system is closely connected to the brain and nervous system. Once immune cells are activated, they release signaling proteins called cytokines. These reach the hypothalamus, a key region of the brain that regulates nerve activity and hormone levels. If the immune system becomes compromised, it may fail to contain these pathogens—leading to illness and disruption of homeostasis (Table 2).

LEM supports this delicate balance by enhancing the function of critical immune cells, including macrophages, B cells, and killer T cells. As a result, the body becomes more capable of detecting, responding to, and eliminating harmful substances. This contributes to overall resilience and helps protect against everyday threats.

Table 2. Diseases caused by bacteria and viruses, etc.

Foreign substances	Examples	Diseases
Bacteria	Staphylococcus aureus, Pseudomonas aeruginosa, Mycobacterium tuberculosis, Escherichia coli, Helicobacter pylori, Streptococcus pneumoniae	Food poisoning, Gum disease, Otitis media, Cystitis, Stomach ulcers, Pneumonia, Pyelonephritis, Tuberculosis
Viruses	Coronavirus, Rotavirus, Norovirus, Influenza Virus	Colds, Flu, Measles, Pneumonia, Herpes Labialis, Chickenpox, Shingles, Herpes Zoster, Cancer
Allergens	Mite, Pollen, Egg, Buckwheat, Latex, Metal, Pet hair, Venicillin, Mold	Asthma, Atopic dermatitis, Urticaria, Hand eczema, Rhinitis, Conjunctivitis
Chemicals, etc.	Asbestos, Cadmium, Cyclosporin, Radiation	Polyps, Cancer

2. Activation of Immune Cells by LEM

2-1. Boosting Phagocytic Activity of Macrophage

Macrophages are a type of white blood cell that function as the body's internal cleaners. They help eliminate invading bacteria, dead cells, and other waste-like substances. In addition, they play a key role in alerting the immune system by presenting foreign materials, known as antigens, to lymphocytes, which triggers further immune responses.

Experiments using latex beads showed that LEM can enhance macrophage activity. For example, when treated with LAP1 (Polysaccharide component of LEM), 67.8% of macrophages absorbed one or more beads, compared to only 37.7% in the control group. Even when measuring uptake of 10 or more beads, LAP1 still showed a significant boost of 44.6% versus just 15.1% in the control group. These findings suggest that LEM has the potential to boost immune defenses by promoting macrophage-mediated phagocytosis. (Table 3).

Table 3. LEM-Induced Enhancement of Macrophage Phagocytosis of Latex Beads

	Percentage of Bead-Phagocytosing Macrophages		
	0	1-9	More than 10
Control	62.3	22.6	15.1
LAP1	32.3	23.2	44.6

Macrophages: Isolated from LAP1-treated and untreated BALB/c mice (10-wk-old, ♂)

Ref. Matsumura H. et al. Journal of the Japan Society of the Reticuloendothelial System 27. 201-207.1987.

2-2. Enhancing Antibody Production

Antibodies are essential for protecting the body against allergens like bacteria, viruses, and even mites.

In lab tests using human blood cells, researchers studied how LEM affects antibody-producing cells. The results were striking: when 200 μg/mL of LEM was added, the number of plaque-forming cells (a marker for antibody production) increased to 171.6%, compared to 100% in the untreated group. This suggests that LEM may help the body produce more antibodies, offering stronger immune defense.

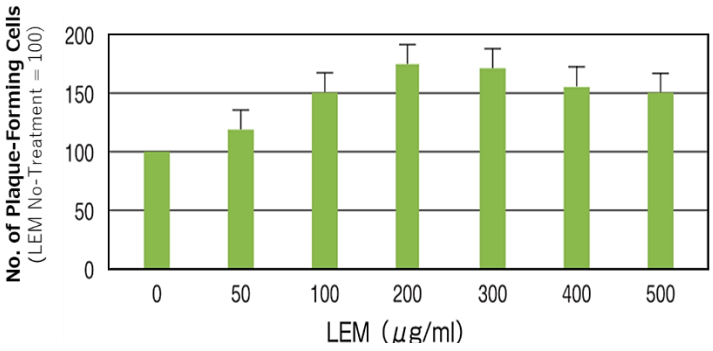


Fig. 5. Enhancement of antibody production by LEM

Ref. Mizoguchi Y. et al. Kan-Tan-Sui 15. 127-135.1987.

Chapter 4: LEM and Its Role in Eliminating Unwanted Substances

1. LEM's Protective Effects Against Bacterial Infections

The development of antibiotics, beginning with penicillin, has had a powerful impact on the treatment of bacterial infections. However, excessive use of these drugs has contributed to the rise of resistant strains, which has created serious new challenges. In addition, humanity continues to face emerging diseases such as Ebola and COVID-19, along with the reappearance of infections like tuberculosis and cholera that were once thought to be under control.

Research has shown that LEM helps enhance bactericidal activity. In experimental studies, mice were pretreated with LEM before being infected with *Pseudomonas aeruginosa* or *Salmonella* spp. After 24 hours, the number of viable bacteria in the spleen and peritoneal cavity was significantly reduced (Fig. 6, 7). These results suggest that LEM activates macrophages in the spleen and peritoneal cavity, enabling them to vigorously engulf the invading bacteria.

Notably, *Pseudomonas aeruginosa* is known for its strong resistance to antibiotics and often causes opportunistic infections in elderly individuals or those with weakened immune systems. Meanwhile, *Salmonella* spp.is a major cause of foodborne illness.

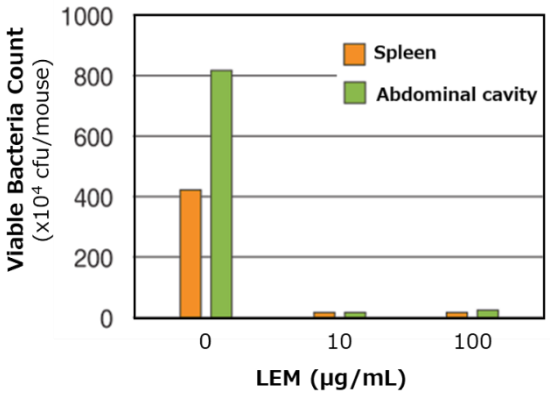


Fig. 6. Immunologically Enhanced Bactericidal Activity of LEM Against *Pseudomonas aeruginosa*

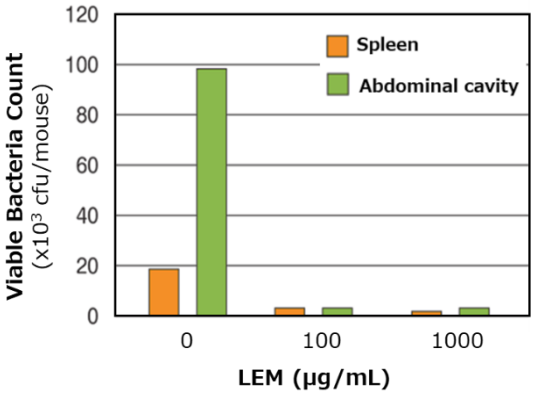


Fig. 7. Immunologically Enhanced Bactericidal Activity of LEM Against *Salmonella* spp.

Ref. Matsumura H. et al. Journal of the Japan Society of the Reticuloendothelial System 27. 201-207.1987.

2. LEM's Antiviral Properties

Viruses are microscopic particles composed of nucleic acid (DNA or RNA) and protein, invisible without an electron microscope. Unlike bacteria, viruses cannot replicate on their own. Instead, they invade animal or plant cells, hijacking cellular functions to multiply. Viral diseases include herpes, colds and influenza, hepatitis, AIDS, and leukemia.

Because antibiotics are ineffective against viruses, treatment typically involves antiviral agents tailored to each specific virus.

2-1. Inhibition of Viral Replication by LEM

- **Method**: Kidney cells from African green monkeys were infected with various viruses and cultured in the presence of water-soluble lignins extracted from LEM.
- **Findings**: This setup revealed strong inhibitory effects on the replication of herpesvirus and Western equine encephalitis virus, and weaker effects on poliovirus, mumps virus, and measles virus.
- **Implication**: As this experiment did not involve white blood cells, the results suggest that LEM's antiviral effects stem from directly acting on the viruses themselves.

Table 4. Antiviral Effects of Water-Soluble Lignins

Virus	Level of Inhibition
Herpes	Strong
Western Equine Encephalitis	Strong
Poliovirus	Weak
Mumps	Weak
Measles	Weak
Cowpox	Not effective

Ref. Sorimachi K. et al. Agri. Biol. Chem. 54, 1337. 1990.

2-2. Effects on Influenza Viruses

In Japan, influenza commonly spreads during the winter season (late November to March). Unlike the common cold, flu symptoms are often abrupt and severe, including high fever (above 38°C), chills, and fatigue. Infants, older adults, and those with chronic respiratory or cardiac conditions are particularly vulnerable, and in some cases, complications can be fatal.

Influenza outbreaks are caused by three types of influenza viruses: A, B, and C. Past pandemics have included the Spanish Flu (1918), Asian Flu (1957), Hong Kong Flu (1968), and the 2009 H1N1 pandemic.

Due to their high mutation rates, viruses frequently evolve, giving rise to new strains. In 2002, a coronavirus originating from wild animals mutated and spread to humans, leading to severe symptoms. This included outbreaks of SARS (spread by bats, 2002–2003) and MERS (spread by camels, 2012–present), both of which posed ongoing global health concerns.

a) LEM's Preventive Action Against Influenza Virus Infection

Kidney cells were treated with various preparations of LEM and then exposed to type A influenza virus. After several days of incubation, the cells were stained and examined for plaques, which are spots that indicate viral damage. The presence of LEM significantly reduced the number of plaques, suggesting that it helped prevent the virus from entering cells and inhibited its replication. These findings indicate the preventive potential of LEM against influenza virus infection.

b) LEM's Inhibitory Action in a Mouse Model

Mice were inoculated with type A influenza virus through their nasal passages, and simultaneously received LEM either nasally or orally. In the group treated with LEM nasally, 50% recovered from the infection. In contrast, 90% of the control group had died by day 13. In the oral administration group, the median survival time extended to 13 days compared to just 9 days in the control. These results suggest that LEM contributed to reducing the severity and spread of infection in this model.

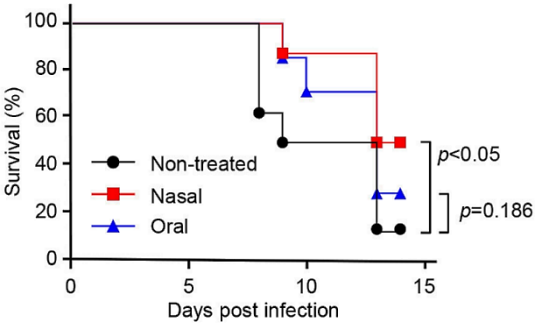


Fig. 8. LEM Treatment Enhances Survival Following Influenza Infection in Mice

c) LEM's Suppression of Viral Replication

MDCK cells (derived from mouse kidney) were exposed to LEM prior to infection with type A influenza virus. Seven hours after infection, intracellular RNA and mRNA were measured. LEM showed a pronounced suppression of both, indicating it inhibits the replication of influenza virus genetic material within cells.

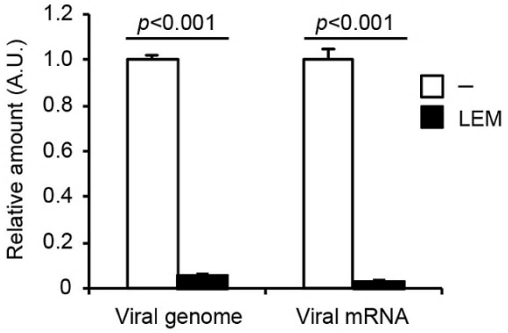


Fig. 9. Suppression of Influenza Virus Replication by LEM

Ref. Kuroki T. et al. Frontiers in Microbiology 9. 1164. 2018.

◆ Summary of Findings

The research revealed three key anti-influenza actions of LEM:

- **Inhibition of Viral Entry**: LEM blocked viral penetration into cells
- **Replication Suppression**: LEM suppressed viral growth within infected cells
- **Protective Effects on Animal Models**: LEM reduced infection severity and boosted survival in animal models

➢ Basic Information on COVID-19

Coronaviruses are a group of viruses that often cause common colds, which most people recover from in about a week. However, when these viruses infect wild animals and then change (mutate), they can sometimes cause more serious illness when transmitted to humans.

COVID-19 is a disease caused by a new kind of coronavirus. It was first identified in Wuhan City, Hubei Province, China, in December 2019. The virus spread quickly across the world, and by June 2020, over 7 million people had been infected globally.

According to Japan's Ministry of Health, Labour and Welfare, most people who contract COVID-19 in Japan have mild symptoms and recover with observation alone. However, around 20% of patients may develop pneumonia and require hospital care. In about 2–3% of cases, patients need treatment in intensive care units, and the illness can be life-threatening, especially for older individuals or those with existing conditions such as diabetes or cardiovascular disease.

3. Anti-Tumor Properties of LEM

3-1. Cancer and Japanese occurrence

Cancer is currently the number one cause of death in Japan, and it's a condition that many people may face during their lifetime—statistically, about one in two Japanese individuals will be diagnosed with some form of cancer. According to data from the Ministry of Health, Labour and Welfare, there are an estimated 3 million cancer patients in Japan, and in 2017, around 980,000 people were newly diagnosed (560,000 men and 420,000 women).

Most early-stage cancers can now be effectively treated or even cured thanks to ongoing advancements in medical science. In more advanced cases, treatment typically involves a combination of radiation therapy, chemotherapy, surgery, hyperthermia and immunotherapy, each selected according to the patient's condition.

Looking at outcomes, the overall 5-year survival rate for cancer cases diagnosed between 2009 and 2011 was 68.4%, and the 10-year survival rate (based on diagnoses from 2003 to 2006) reached 57.2%. These numbers have steadily improved over time, likely due to new cancer medications and refined surgical techniques that help manage the disease more effectively.

In 2018, Dr. Tasuku Honjo, a Distinguished Professor at Kyoto University, received the Nobel Prize in Physiology or Medicine for a breakthrough in cancer immunotherapy. He discovered a molecule called PD-1 that functions as a brake on immune cells. Cancer cells can exploit this mechanism, making it harder for the immune system to respond. His research contributed to the development of immune checkpoint inhibitors, a novel class of drugs that help restore immune function. These therapies have produced remarkable results in patients who previously gained little from conventional cancer treatments.

Table 5. Cancer Survival Rates in Japan: 5-Year and 10-Year Relative Estimates by Site

Cancer Site	5-Year Survival Rate (%)	10-Year Survival Rate (%)
Prostate	100.0	100.0
Breast (Female)	93.7	85.9
Thyroid	92.6	88.3
Cervix	86.4	74.1
Bladder	79.2	71.6
Uterus	76.8	69.0
Colon	76.0	70.4
Kidney	72.1	61.7
Rectum	69.9	66.3
Stomach	66.9	56.5
Lung	40.8	32.0
Liver	40.1	15.0
Pancreas	12.6	3.0

National Cancer Center Hospital. 2020.3.

3-2. Antitumor Action of LEM

LEM has demonstrated antitumor properties through two key mechanisms: by activating immune cells and by directly inducing cancer cell death.

a) Antitumor Activity Against Ascites Hepatoma Cells (AH414)

In this study, hepatoma cells were transplanted into the ascites fluid of rats. On Day 8, the number of cancer cells present in the fluid was measured and compared across groups. The control group received saline, while the treatment groups received either 50 mg or 100 mg of LEM per animal.

- **Results**

The group treated with LEM showed notable suppression of tumor growth. At a dose of 50 mg, cancer cell proliferation was reduced to 52%, and at 100 mg, it decreased to 22% (Table 6). Photograph 3 captures a marked increase in lymphocytes and the presence of cancer cells undergoing destruction following LEM administration.

Table 6. Effect of LEM on Ascites Hepatoma Cell Growth

LEM Dose (mg)	Hepatoma Cell Number ($\times 10^7$/ml)	Growth Rate (%)
0	16.8	100
50	8.8	52
100	3.7	22

Ref: Sugano et al. Cancer Letters. 17. 109–114. 1982

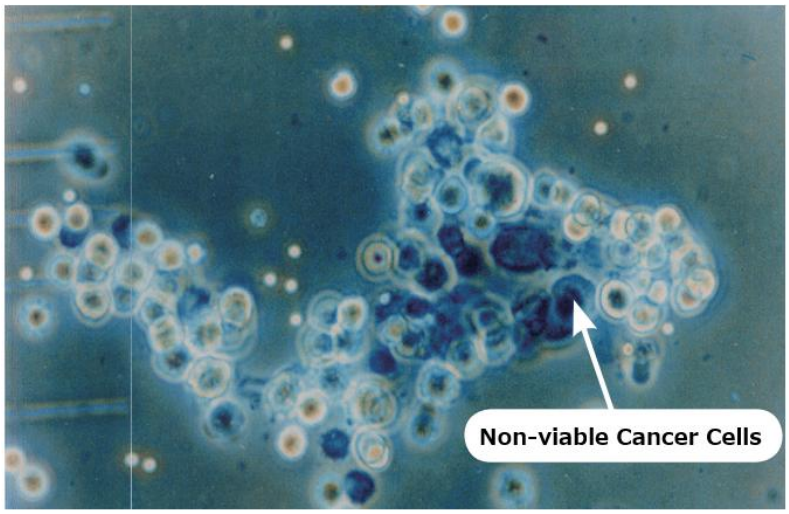


Photo. 3. Cancer cell death induced by LEM administration
Supplied by Nobuhiko Kanno, PhD, Professor Emeritus at Toyama Medical and Pharmaceutical University

b) Inhibitory effect on cancer induction by chemical carcinogens

Further trials involved repeated administration of LEM at doses of 700 mg/kg and 100 mg/kg, each given 15 times. After 41 weeks, rats in the LEM-treated group exhibited liver weights that were approximately half those of the untreated group. This reduction indicates an inhibitory effect on chemically induced hepatocarcinogenesis (Sugano et al. Cancer Letters. 17. 104–114. 1982). A broader summary of LEM's antitumor performance across animal models and human clinical observations is provided in Table 7. LEM was found to suppress cancer cell growth, reduce chemically induced carcinogenesis, and prevent metastasis.

Notably, in clinical settings, postoperative intake of LEM showed promise in lowering the risk of recurrence in patients with uterine cancer.

Table 7. Anti-Tumor Properties of LEM

Study Subject	Model Organism	Observed Effect
Proliferation inhibition of ascites hepatoma cells AH414	Rat	Suppression of cell proliferation by ≤50%
Proliferation inhibition of ascites hepatoma cells AH60C	Rat	Suppression of cell proliferation by ~60%
Inhibition of carcinogenesis induced by 3'-Me-DAB	Rat	Carcinogenesis reduced by ~40%
Inhibition of colon cancer induced by azoxymethane	Rat	Oral administration led to 63% inhibition
Inhibition of RL1 tumor metastasis	Mouse	Metastasis suppressed by 50%
Postoperative recurrence of uterine cancer [*1]	Human	Among 13 surgical cases, 12 experienced recurrences. One case showed tumor reduction and CA-19-9 decrease. Recurrence confirmed in multiple cases post-surgery.

[*1] Kohama. 6th Annual Meeting of the Japan Society for Complementary and Alternative Medicine, Sendai, 2003.

c) LEM Suppresses Melanoma Progression via Improvement of the Tumor Microenvironment Altered by Immune Editing

It is known that cancer cells attract regulatory T cells (Treg cells), which are immune modulators, to their surroundings in order to suppress cellular immunity, including the activity of killer cells. This mechanism contributes to tumor proliferation and is referred to as cancer immune editing. To investigate the effect of LEM on tumor proliferation through this process, we conducted a study using the melanoma cell line B16BL6.

Melanoma cells were transplanted into the footpads of mice, and tumor progression was monitored over time. The mice were divided into three groups: one group received a diet supplemented with 1% LEM, another received 2% LEM, and the third served as a control group with no LEM supplementation. Tumor size was measured at multiple time points, and the composition of splenic immune cells, particularly Treg cells, was assessed.

The results showed the following (Fig. 10, 11):

- Tumor size was reduced in both the 1% and 2% LEM groups compared to the control group.
- Tumor growth was inhibited in a concentration-dependent manner.
- In mice inoculated with B16BL6, the proportion of Treg cells (Treg/CD4$^+$) in the spleen was decreased following LEM intake.

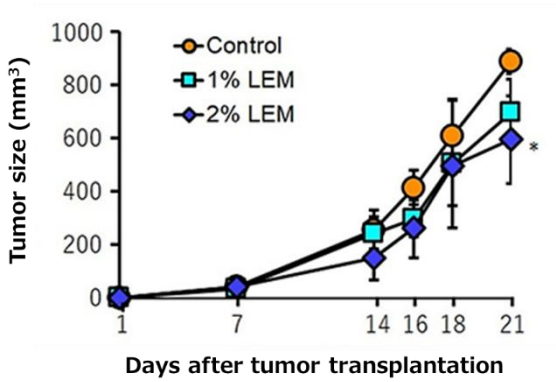


Fig. 10. Effect of LEM on Tumor Progression Over Time

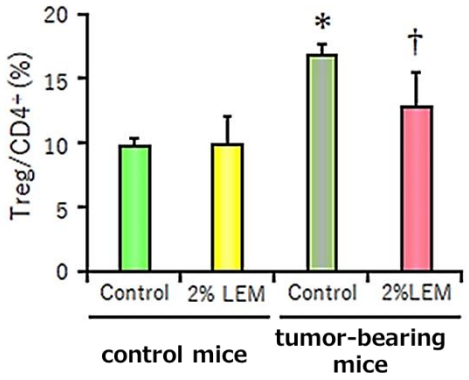


Fig. 11. LEM-Induced Changes in Treg Cell Frequency (Treg/CD4$^+$) in the Spleen

Ref. Josai University and Noda Shokukin Noda Shokukin Kogyo 2017.

d) Direct Antitumor Effects of LEM on Leukemia Cells

Leukemia is a type of blood cancer that can be classified as either acute or chronic, and further divided into myeloid and lymphoid forms. In Japan, myeloid leukemia tends to be more common among adults, while lymphoid leukemia occurs more frequently in children (Table 8).

Recent studies have shown that LEM has the ability to arrest the growth of leukemia cells. When human chronic myeloid leukemia cells (K562) were cultured with LEM or with its water-soluble lignin fraction, both treatments significantly suppressed leukemia cell proliferation (Fig. 12).

This outcome appears to be the result of a direct inhibitory effect on leukemia cell division, caused by LEM or its lignin-derived components, rather than being mediated by immune cell activity.

Table 8. Classification of Leukemia

Type	Characteristics	Representative Subtypes
Acute Leukemia	● Rapid proliferation of leukemia cells ● Increase of immature, non-functional cells	● Acute Myeloid Leukemia (Most common in Japan) ● Acute Lymphoblastic Leukemia (Common in children)
Chronic Leukemia	● Slow proliferation of leukemia cells ● Increase of mature, functional cells	● Chronic Myeloid Leukemia (~20% of cases) ● Chronic Lymphocytic Leukemia (Rare in Japan)

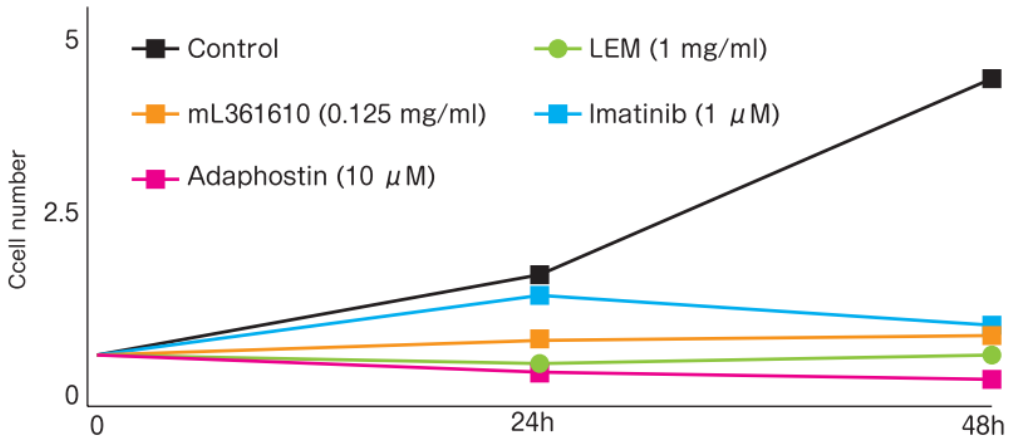


Fig. 12. Inhibitory Effects of LEM on Cell Proliferation in Chronic Myelogenous Leukemia

mL361610: Water-soluble lignins fraction, imatinib: Approved therapeutic drug for leukemia, Adaphostin: Investigational therapeutic candidate for leukemia.

Chapter 5: Anti-Allergic Action of LEM

1. Classification of Allergic

Allergic diseases are among the most common conditions affecting people in Japan. These disorders result from an overactive immune system responding to external factors such as pollen, dust mites, and certain foods, and self-components. There are four primary types of allergic reactions (Table. 9). Type I and IV allergies are more likely to develop when exposure to antigens (allergens) and stress are added to one's constitution (genetic factors). Autoimmune diseases (classified as Type II and Type III allergies) are typically triggered by infections, female hormones, and excessive stress, in addition to inherited susceptibility. Infectious agents, in particular, play a significant role in both the onset and progression of these conditions. For this reason, maintaining a healthy lifestyle with balanced nutrition and sufficient sleep is essential for both the prevention and management of allergic conditions.

Table 9. Classification and Characteristics of Allergic Diseases

Classification	Characteristics	Main Antibodies or Effectors	Representative Diseases
Type I	Immediate-type hypersensitivity	IgE	Hay fever, Atopic dermatitis, Bronchial asthma, Urticaria, Allergic conjunctivitis
Type II	Cytotoxic-type autoimmune diseases	IgM, IgG	Hemolytic anemia, Basedow disease, Hashimoto's disease, Infertility, Recurrent pregnancy loss, Idiopathic thrombo-cytopenic purpura
Type III	Immune complex-type autoimmune diseases	IgM, IgG	Rheumatoid arthritis, Ulcerative Colitis
Type IV	Delayed-type hypersensitivity	Sensitized lymphocytes	Hand eczema, Rash, Metal allergy

1-1. Mechanism of Type I Allergy Development

When an allergen enters the body, it prompts the production of IgE antibodies. Upon subsequent exposure to the same allergen, mast cells release granules containing chemical substances such as histamine and proteolytic enzymes. These mediators cause common allergic symptoms including hives, sneezing, and asthma（Fig. 13）.

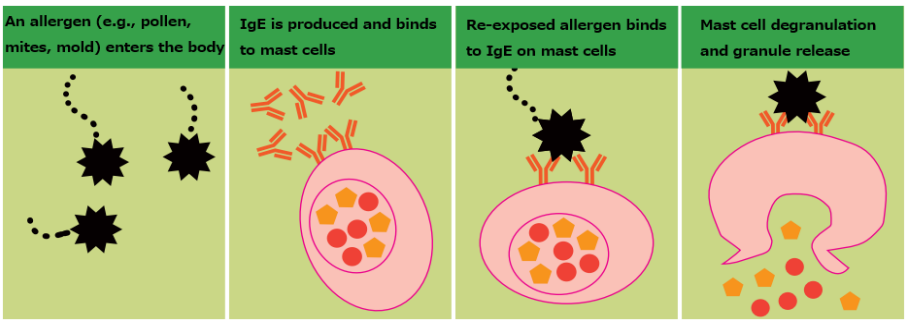


Fig. 13. Scheme of IgE-Mediated Allergic Reaction

2. Inhibition of Type I Allergy by LEM

2-1. Inhibition of Hyaluronidase by LEM

- **Method**

After the addition of hyaluronidase solution to the solution of LEM dissolved in buffer solution, hyaluronic acid substrate solution was added. Following cessation of the reaction, a chromogenic agent was added, and the absorbance at 585 nm was measured to calculate the inhibition rate (Table 10).

- **Results**

LEM exhibited inhibitory activity against hyaluronidase. This enzyme is known to be involved in the process of histamine release, which contributes to allergic symptoms.

Table10. LEM's effectiveness in reducing hyaluronidase activity

LEM (mg/ml)	Inhibition (%)
5.0	27.4
7.5	74.0
10.0	100.0

Ref: Kohayakawa S et al. New food industry 60. 42-48. 2018.

2-2. Inhibition of β-Hexosaminidase Release by LEM

β-Hexosaminidase is an enzyme contained within mast cell granules. Like hyaluronidase, it is released alongside histamine during allergic responses. Histamine is responsible for symptoms such as itching, swelling, and sneezing.

Studies demonstrated that LEM suppressed the release of β-hexosaminidase (Table 11).

Table11. LEM's effectiveness in reducing β-Hexosaminidase Release

LEM (μg/ml)	Inhibition (%)
257	14.8
514	33.1
1028	54.7
2055	79.4

Ref: Kohayakawa S et al. New food industry 60. 42-48. 2018.

◆ **Conclusion**

LEM was found to inhibit two allergy-related enzymes, hyaluronidase and β-hexosaminidase. By limiting their activity, LEM may help reduce histamine release, thereby contributing to the alleviation of allergic symptoms (Fig. 14).

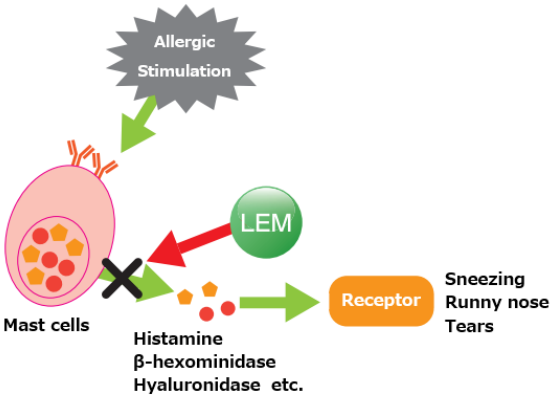


Fig. 14. LEM's mechanism of action in modulating histamine response

3. Clinical Study of LEM for Atopic Dermatitis

A clinical study was conducted at a dermatology hospital in Higashikurume, Tokyo to evaluate the effects of LEM on atopic dermatitis. A total of 21 patients were orally administered with a test product containing LEM, as outlined in Table 12, over a period of three months. During the trial, the use of steroid ointments was discontinued for all participants. Only vitamin supplements and eucalyptus oil were used in combination with LEM.

Table 12. LEM Dosage and Administration Period

Target Group	Experimental Material	Dosage (packs/day)	Administration Period (months)
Adults	LEM 3 g/pack	2	3
Adolescents (13–18 years)	LEM 0.72 g/pack Lactose 2.28 g/pack	2–3	3
Children (infants to 12 years)	LEM 0.32 g/pack Dietary Fiber 1.08g Oligosaccharide 0.6g	2–3	~3

- **Results**

Remarkably, a total of 19 patients (91%) showed improvement ranging from 1 to 3 points (Table 13). Among them, 4.8% improved by 3 points, 38.1% improved by 2 points, and 47.6% showed a 1-point improvement (Table 13, Fig. 15). One particularly notable case demonstrated a reduction in IgE levels, decreasing from 1,145 at the beginning of the trial to 662 by day 91. Photograph 4 presents an example demonstrating the observed therapeutic improvement.

Before dosing **70 days after dosing**



Photo. 4. Improvement in Atopic Dermatitis Through LEM Intake

Table 13. Clinical Trial Results of LEM for Severe Atopic Dermatitis

Improvement Level by Score

Improvement Score	Number of Cases	Improvement Rate (%)
3-point improvement	1	4.8
2-point improvement	8	38.1
1-point improvement	10	47.6
No improvement	2	9.5

Number of Patients: 21

Administration Period: 3 months (during administration, steroid reduction and discontinuation)

Date: May 2014

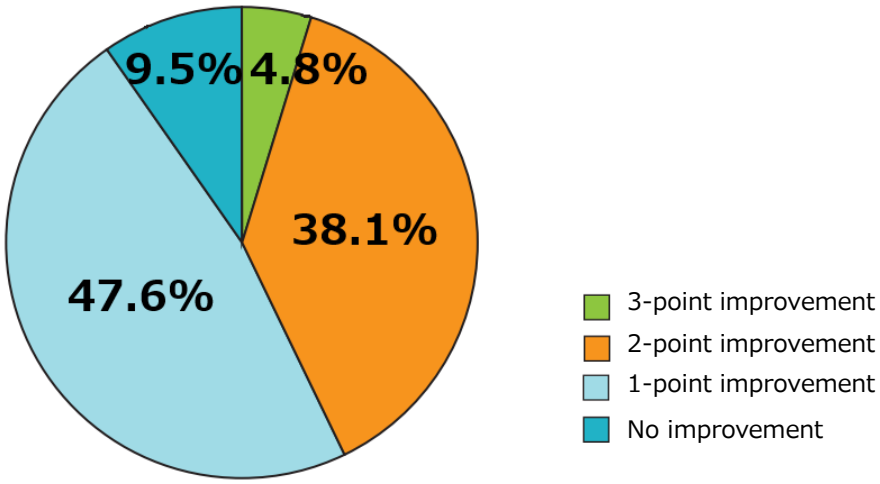


Fig. 15. Clinical Response Distribution Following LEM Administration

4. Suppression of Autoantibody Production by LEM

Autoimmune diseases such as rheumatoid arthritis, Graves' disease, ulcerative colitis, and certain infertility-related conditions affect an estimated 8 million people in Japan, including mild cases that often go unnoticed. These conditions stem from immune system imbalances, causing the body to produce autoantibodies that trigger inflammation and a wide range of symptoms.

Recent observations highlighted LEM's potential to counter these reactions. As illustrated in Table 14, LEM helped suppress the production of inflammation-inducing autoantibodies, suggesting a promising role in supporting immune balance and improving quality of life for individuals affected by autoimmune disorders

Table 14. Suppressive Effect of LEM on Autoantibody Production

No.	Patient	Disease/Symptom	Duration (Months)	Course/ Outcome
1	30 y/o, Female	Hyperprolactinemia, 3 miscarriages	3	Anti-cardiolipin Ab: negative, Delivery
2	29 y/o, Female	Infertility	2	Anti-phospholipid Ab: negative, Delivery
3	36 y/o, Female	Late miscarriage, yellow-green discharge	3	Anti-phospholipid Ab: from 320 to 20, Decreased
4	30 y/o, Female	1 miscarriage	3	Anti-cardiolipin Ab: from 80 to 20, then negative, Decreased
5	29 y/o, Female	Allergic rhinitis, 1 miscarriage	3	Anti-phospholipid Ab: negative, Delivery
6	31 y/o, Female	Chocolate cyst	4	Anti-phospholipid Ab:from 160 to 40, Decreased
7[*1]	29 y/o, Female	Systemic lupus erythematosus	4	Anti-DNA Ab: from 19 to 7, Decreased

[*1]: prednisone was used in combination

Ref.: Kasai S. Biodefense Research Association Bulletin. March 1999.

Chapter 6: Effects of LEM on Lipid Metabolism

Cholesterol plays an essential role as a structural component of cell membranes and is distributed throughout the body, including in the blood, brain, internal organs, and muscles. Elevated cholesterol levels in the blood are known to contribute to serious conditions such as arteriosclerosis, myocardial infarction, and cerebral infarction. In Japan, the number of individuals diagnosed with dyslipidemia has been rising, a trend closely linked to the westernization of dietary habits. As of 2009, more than 14 million people were identified as having suspected metabolic syndrome, including those in the pre-metabolic stage.

Cholesterol-Lowering Effect of LEM

- **Methods**

Male white rats were provided with a cholesterol-enriched diet containing LEM. After two weeks, blood and liver samples were collected and analyzed to determine cholesterol levels.

- **Results**

The inclusion of LEM in the diet was found to suppress the rise in serum cholesterol levels (Table 14). This effect is thought to result from inhibited absorption of cholesterol and bile acids in the intestinal tract. No significant difference was observed in liver cholesterol levels.

Table 14. Cholesterol-Lowering Effect of LEM

Group	Serum Cholesterol (mg/100 mL)	Liver Cholesterol	
		mg/g	mg
(I) Diet 1			
Control + Cholesterol	92 ± 6	32 ± 5	336 ± 49
1% LEM + Cholesterol	80 ± 15	28 ± 3	317 ± 65
(II) Diet 2			
Control + Cholesterol	158 ± 86	34 ± 5	213 ± 45
0.1% LEM + Cholesterol	130 ± 35	36 ± 5	209 ± 62
0.5% LEM + Cholesterol	108 ± 51	38 ± 11	217 ± 58

Diet 1: Casein feed + casein feed, diet 2: Casein feed

Ref. Ohta T. et al. The Technical Bulletin of Faculty of Horticulture, Chiba University. No. 24. p.11–17. 1976.

Chapter 7: Cytoprotective Effects of LEM

1. LEM Protects Liver

Immune responses targeting liver cells are thought to contribute to the development and progression of chronic hepatitis. Experimental studies have shown that antibodies and activated macrophages can damage liver cells, impairing their function. In contrast, clinical investigations suggest that LEM may help slow the progression of chronic hepatitis and promote the recovery of liver function. Based on these findings, the mechanism by which LEM protects liver cells was examined.

To assess the protective effect of LEM, liver injury was induced in rat liver cells using activated macrophages. The liver cells were pre-treated with LEM, and its impact on protein synthesis was evaluated. Pre-treatment with LEM significantly increased protein synthesis in a concentration-dependent manner and prevented the decline typically caused by macrophage-induced injury. These results clearly indicate that LEM exerts a protective effect on liver cells (Fig. 16).

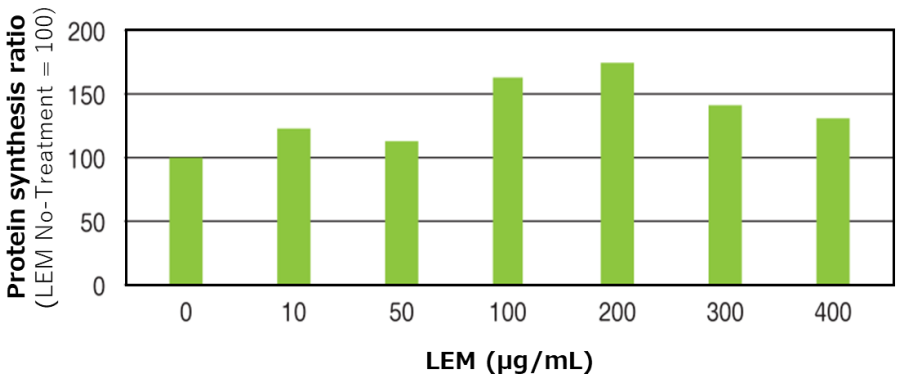


Fig. 16. Protective effect of LEM against liver cell injury

Ref. Mizoguchi Y. et al. Kan-Tan-Sui 15. 127-135.1987.

2. Neuroprotective Action of LEM

In Japan, stroke is currently the third leading cause of death. It includes cerebral infarction, subarachnoid hemorrhage, and cerebral hemorrhage (Fig. 17), and results in approximately 130,000 deaths each year. Among these, cerebral infarction is the most common, accounting for about 70% of cases. Cerebral hemorrhage accounts for 20 to 25%, while subarachnoid hemorrhage represents only a few %.

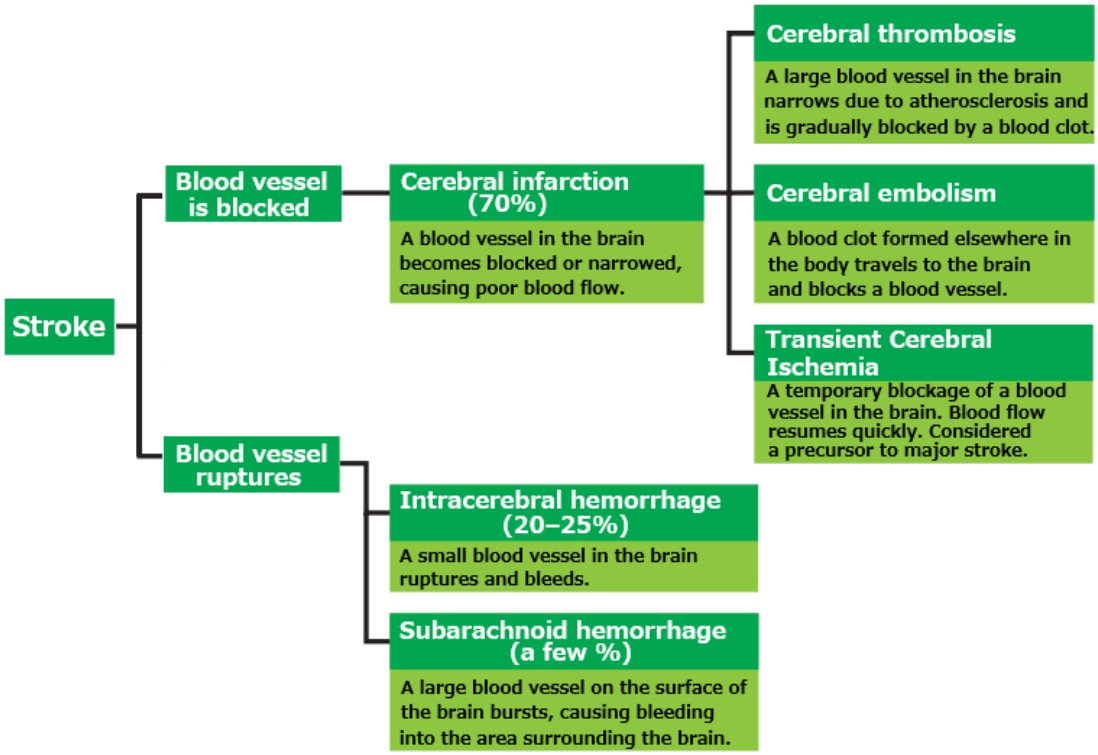


Fig. 17. Types of Stroke

Before a serious stroke occurs, temporary symptoms such as hemiplegia, numbness, and speech impairment may appear. These symptoms typically disappear within 24 hours. When blood flow resumes, reactive oxygen species are locally generated. This leads to secondary damage associated with ischemia, which contributes to the spread of neurological disorders.

To evaluate the inhibitory effect of LEM on reactive oxygen species-induced damage in the ischemic periphery, a mouse model of hypoxic cerebral ischemia was used. This model is considered representative of human cerebral infarction.

- **Methods**

Mice were fed a diet containing LEM for two weeks. Under anesthesia, the right common carotid artery was double ligated. Three hours later, the mice were exposed to hypoxic gas for 30 minutes. After 24 hours of recovery, brain damage was assessed by measuring the volume of cerebral infarction and performing tissue staining.

• **Results**

The volume of cerebral infarct foci was significantly reduced in the LEM-treated group (Fig. 18). In addition, infarct formation in the LEM group was limited to specific regions (Fig. 19). These findings suggest that LEM has a protective effect against neuronal damage caused by reactive oxygen species.



Fig. 18. Suppression of Cerebral Infarct Nest Formation by LEM

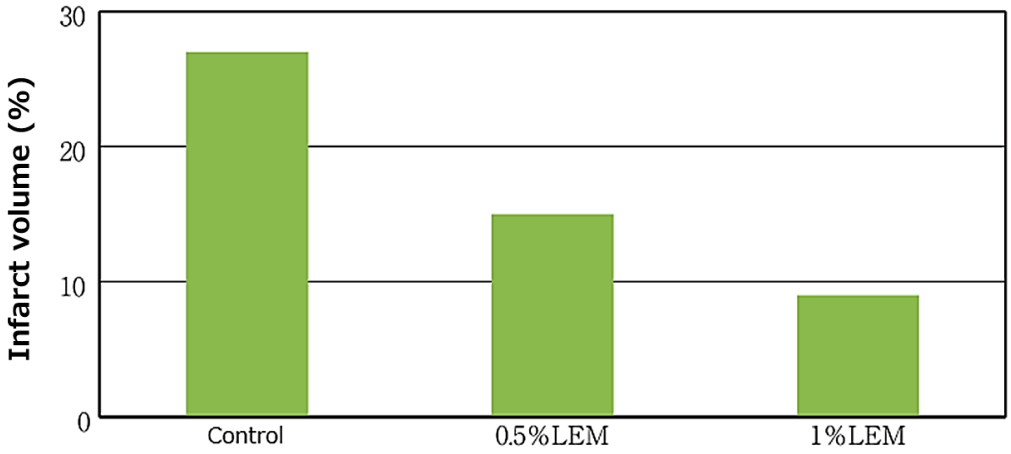


Fig. 19. Suppression Rate of Cerebral Infarct Foci Area by LEM

Ref. Xuan M. et al. The Japanese Society for Complementary and Alternative Medicine 8. 99-107. 2011.

Chapter 8: LEM's Effect on the Autonomic Nervous System

The autonomic nervous system plays a central role in regulating essential body functions, including digestion, respiration, circulation, excretion, and reproduction. It operates independently of conscious control and is composed of two branches: the sympathetic and parasympathetic nervous systems. These nerves are distributed throughout the body and help coordinate the activity of internal organs. When the balance between these two systems is disrupted, individuals may experience a range of symptoms such as fatigue, dizziness, shortness of breath, palpitations, and headaches. These are often referred to as indefinite complaints, meaning that no specific underlying disease can be identified even after medical examination.

These autonomic functions are closely integrated with the immune and endocrine systems, forming a dynamic regulatory network that maintains physiological balance and responds adaptively to internal and external stressors (Fig. 20).

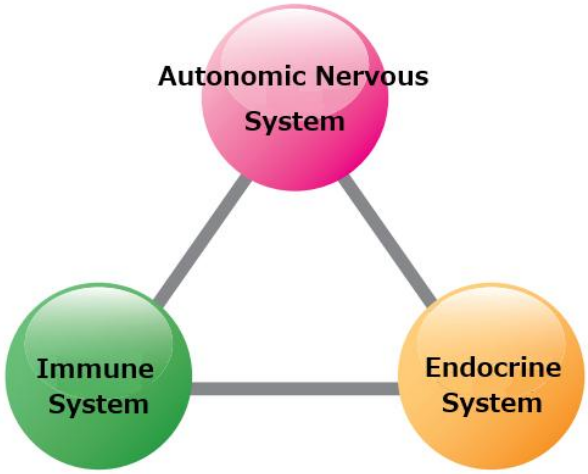


Fig. 20. Interaction Among the Autonomic Nervous, Immune and Endocrine Systems

In a study involving 150 cases, LEM was administered alongside Chinese herbal medicine tailored to each patient's condition. The following observations were made:

1. The combination of LEM with appropriate Kampo formulations enhanced the reliability of therapeutic outcomes.
2. Continued intake of LEM helped prevent the recurrence of symptoms, even after Kampo treatment was discontinued.

*Note: Kampo formulations used in this study included Kamishoyosan, Hangekobokuto, Shibakokeishito, Ryubanshashito, and others (Sanekata T. Biodefense Research Association Newsletter, No. 13, August 1991 in Japansese).

Chapter 9: LEM Clinical Applications

Many studies have explored the mechanisms underlying the immune-modulating effects of LEM. Building on these foundational findings, its impact on human health is now being actively investigated.

1. Clinical Investigation of LEM in Chronic Hepatitis

1.1 Chronic Hepatitis B

The liver is often referred to as a “silent organ” because it rarely shows symptoms, even when significantly damaged. Nonetheless, it can be affected by viral infections, excessive alcohol intake, or certain medications.

In Japan, viral hepatitis accounts for approximately 70 to 80% of all liver diseases, with most of the remaining cases attributed to alcohol-related damage. The primary viruses responsible for liver injury include hepatitis A, B, and C (Table 16).

Hepatitis A does not lead to chronic infection, but hepatitis B and C may progress to chronic hepatitis if the virus persists. In some cases, chronic hepatitis can further develop into cirrhosis or liver cancer.

Table 16. Characteristics of Major Viral Hepatitis

Category	Hepatitis A	Hepatitis B	Hepatitis C
Source of Infection	Contaminated food (especially overseas)	• Mother-to-child transmission (common) • Blood transfusion • Sexual contact	• Blood transfusion • Shared injection equipment • Sexual contact • mother-to-child transmission (rare)
Route of Infection	Oral	Blood	Blood
Course of Hepatitis	Acute hepatitis (occasionally severe), does not become chronic	Infection during childhood often leads to chronic hepatitis	Frequently becomes chronic, may progress to cirrhosis and liver cancer

1.2 LEM Treatment for Chronic Hepatitis B

An open-label study involving 66 patients was conducted by specialists across 16 medical centers nationwide. The results showed notable reductions in viral antigens, increased antibody production, and improvements in liver function. Figure 21 illustrates the degree of liver function improvement, with approximately half of the patients showing marked, moderate, or mild improvement.

A separate study involving 40 additional patients with hepatitis B reported a high seroconversion rate of 25%, indicating a transition to a quiescent state of the disease. Importantly, no adverse effects were observed in any of the 109 cases during the four-month treatment period.

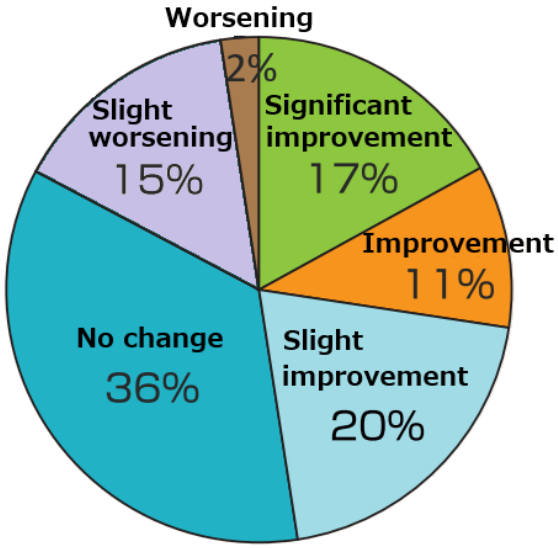


Fig. 21. Improvement of liver function in type B hepatitis by LEM

Ref. Harada T. and Kanetaka T. Kan-Tan-Sui 14. 327-325. 1987.

1-3. Chronic Hepatitis C

Hepatitis C is caused by the hepatitis C virus, which is known for its tendency to become chronic and its frequent progression to cirrhosis and liver cancer (Fig. 22). The virus is classified into four genotypes, labeled I through IV. In Japan, approximately 80% of hepatitis C patients are infected with type II, while about 20% have type III. Interferon α and β therapies are generally considered more effective for type III than for type II.

Following infection with the hepatitis C virus, 60 to 70% of individuals develop chronic hepatitis. Among these, around 40 % will progress to cirrhosis within 15 to 20 years, and approximately 25% will develop liver cancer within 20 to 30 years.

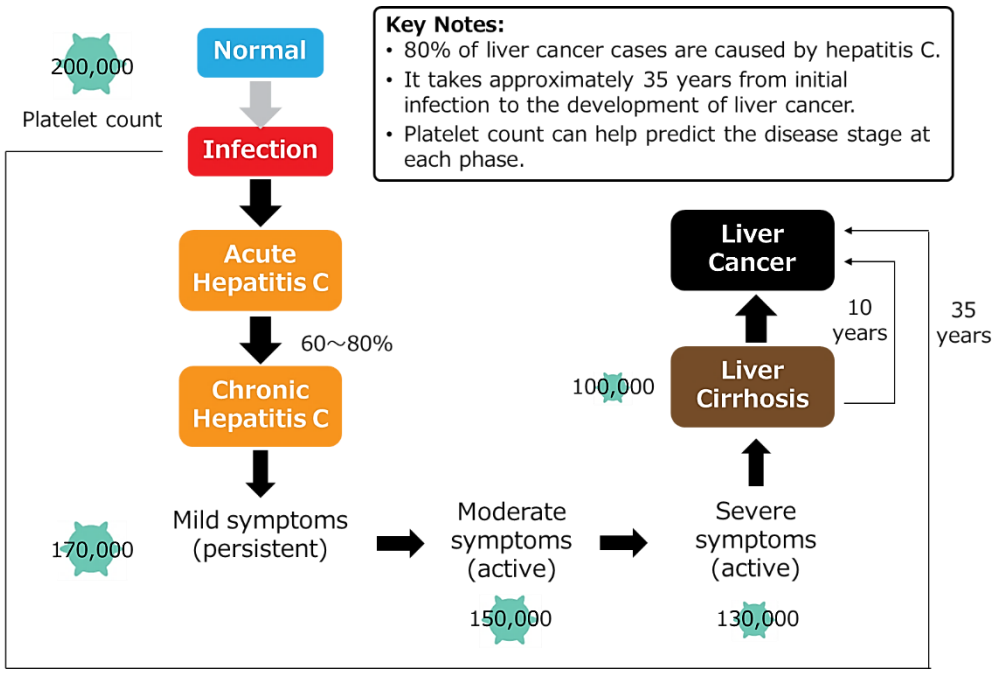


Fig. 22. The Path from Hepatitis C to Liver Cancer

Created based on The Chunichi Shimbun. July 15. 1995

1-4. Effect of LEM on Chronic Hepatitis C and Cirrhosis

Six patients diagnosed with chronic hepatitis C and cirrhosis received treatment with LEM. The group consisted of individuals aged 51 to 70, with an average age of 63, including three males and three females. Each patient was administered 6 grams of LEM per day over a period ranging from 6 to 12 months.

Liver function assessed through AST, ALT, γ-GTP, and ZTT, as well as core antibody levels, HCV-RNA quantification, and AFP levels, was monitored monthly. The results were evaluated based on changes observed before and after LEM administration, and categorized as improvement, no change, or worsening.

Approximately half of the patients showed improvement in hepatocellular damage, along with reductions in hepatitis C virus antibody titers and viral load. These findings suggest that LEM exerts a gradual but meaningful therapeutic effect. No side effects or symptom worsening were reported during the treatment period (Fig. 23).

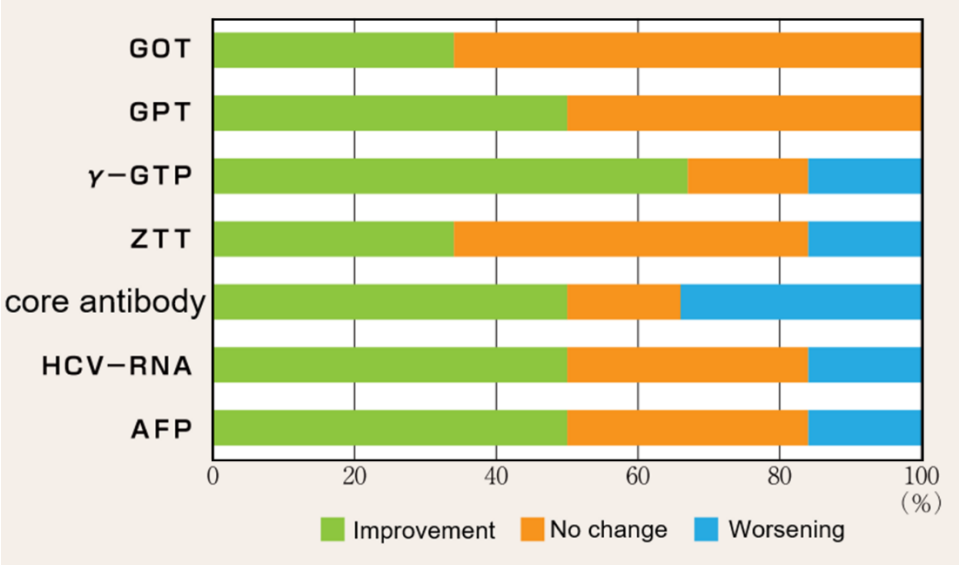


Fig. 23. Effect of LEM on Hepatitis C

Ref. Takashima S. et al. Presentation at the 3rd Annual Meeting of the Japanese Society for Complementary and Alternative Medicine. 2000.

2. Immunological Effects of LEM on HIV-Infected Hemophilia Patients

This study investigated the immune-enhancing effects and clinical outcomes of LEM supplementation in 29 HIV-positive hemophilia patients, including 24 with hemophilia A and 5 with hemophilia B. All participants had acquired HIV through contaminated blood coagulation products and received 6 to 9 grams of LEM daily for a minimum of one year. Among the subjects, 2 were diagnosed with AIDS, 1 was classified as an asymptomatic carrier (ARC), and 26 presented with AIDS-related complex (AC). The study duration extended up to 25 months, during which no antiretroviral drugs such as AZT (Azidothymidine) or other immunomodulators were administered. Immunological assessments included CD4 (helper T-cell) counts, CD4/CD8 ratio analysis, and lymphoblastogenic responses to phytohemagglutinin (PHA), concanavalin A (ConA), and pokeweed mitogen (PWM). The observed efficacy rates exceeded 60%, indicating a notable immune response. Importantly, no adverse effects attributable to LEM were reported throughout the study period.

Ref. Presentation at the 5th Annual Meeting of the Japanese Society for AIDS Research 1991.

3. Preventive Effect of LEM on Recurrence of Endometrial Cancer After Surgery

Thirteen patients diagnosed with endometrial cancer (average age: 61.2 ± 11.4 years) underwent extensive total hysterectomy followed by three monthly courses of chemotherapy consisting of carboplatin, endoxan, and adriamycin. LEM was administered at 6 g/day (3 g x twice daily) for an average duration of 22.5 ± 13.5 months, beginning in the second week after surgery. After hospital discharge, recurrence was monitored every two months using vaginal smears, tumor marker tests, ultrasound imaging, and CT scans. If any signs of recurrence were detected, follow-up examinations were conducted monthly.

Among the 13 cases, 12 showed no signs of recurrence. In one case, an elevation in CA19-9 was observed three months after discharge. However, when the LEM dosage was increased to 9 g/day (administered as 3g x 3 times daily), the marker levels began to decline. These findings suggest that LEM may have a suppressive effect on cancer recurrence in postoperative endometrial cancer patients.

Ref. Kohama T. Presentation at the 6th Annual Meeting of the Japanese Society for Complementary and Alternative Medicine. 2003.

List of LEM Academic Reports

1 Kohayakawa S. et al. (2018) Anti-allergic effects of a water-soluble lignin rich fraction (JLS-18) prepared from the extract of culture medium of *Lentinus edodes* mycelia (LEM). New food industry 60. 42-48. (In Japanese)

2 Kuroki T. et al. (2018) Inhibition of influenza virus infection by *Lentinus edodes* mycelia extract through its direct action and immunopotentiating activity. Frontiers in Microbiology 9. 1164.

3 Xuan M. et al. (2017) Cerebroprotective effects of a water-soluble extract from the culture medium of *Lentinus edodes* mycelia. New food industry 59. 1-14. (In Japanese)

4 Horiuchi S. et al. (2016) Neuroprotective effects of a water-soluble extract from the culture medium of *Lentinus edodes* mycelia (LEM). New food industry 58. 1-10. (In Japanese)

5 Xuan M. et al. (2011) Protective effects of a water-soluble extract from culture medium of *Lentinus edodes* mycelia against neuronal damage after hypoxia-ischemia in mice. The Japanese Society for Complementary and Alternative Medicine 8. 99-107. (In Japanese)

6 Yamamoto Y. et al. (1997) Immunopotentiating activity of the water-soluble lignin rich fraction prepared from LEM -The extract of the solid culture medium of *Lentinus edodes* mycelia. Bioscience, Biotechnology, and Biochemistry 61. 1909-1912.

7 Morinaga H. et al. (1994) An *in vivo* study of hepatic and splenic interleukin-1β mRNA expression following oral PSK or LEM administration. Japanese Journal of Cancer Research 85. 1298-1303.

8 Hibino Y. et al. (1994) Productions of interferon-γ and nitrite are induced in mouse splenic cells by a heteroglycan-protein fraction from culture medium of *Lentinus edodes* mycelia. Immunopharmacology 28. 77-85.

9 Sarkar S. et al. (1993) Antiviral effect of the extract of culture medium of *Lentinus edodes* mycelia on the replication of herpes simplex virus type 1. Antiviral Research 20. 293-303.

10 Kajihara J. et al. (1993) Characterization of antiviral water-soluble lignin from bagasse degraded by *Lentinus edodes*. Holzforshung 47. 479-485.

11 Hattori T. et al. (1993) Formate and Oxalate Esters in Lignin Obtained from Bagasse Degraded by *Lentinus edodes*. Journal of the Japan Wood Research Society 39. 1317-1321.

12 Morinaga H. et al. (1992) Inhibitive effects of LEM by oral administration of AH-60C and RL ♂1 for liver metastasis. Biotherapy 6. 763-765. (In Japanese)

13 Mizoguchi Y. et al. (1992) Effect of extract of cultured *Lentinus edodes* mycelia (LEM) on platelet activating factor synthesis from mouse hepatic sinusoidal endothelial cells. Kampo Medicine 42. 307-311. (In Japanese)

14 Tabata T. et al. (1992) Mitogenic activities of heteroglycan and heteroglycan- protein fractions from culture medium of *Lentinus edodes* mycelia. Immunopharmacology 24. 57-63.

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