

Clinical Trial report on Chronic Hepatitis treatment

Using *IMMUNE-ASSIST* brand mushroom extract mixture in conjunction with the drug Lamivudine [Epivir]

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Abstract: These are the results of a study conducted on 60 patients with hepatitis over a two year period, showing the value of adding proprietary *Immune-Assist* brand of medicinal mushroom polysaccharide mixture to conventional therapy in order to achieve greater effectiveness than can be provided by the conventional therapy alone.

Introduction:

In recent years, it has been found that the polysaccharides extracted from edible fungi have various biological activity functions. The city of Lishui, which lies in the south-west of Zhejiang province, is one of the major mushroom cultivation districts in China and has rich resources of these edible fungi. It is a long-standing tradition for the populace of this area to use the edible fungi as a part of their medicine. When we researched the folk recipes of the people of this area, we found a widespread belief that edible fungi can protect the liver, reduce obesity and improve overall immunity. According to this, we used modern extraction methods and prepared a mushroom polysaccharide mixture according to the

formula of ***Immune-Assist***, a proprietary health supplement developed as a joint project between the Government of Zhejiang province and a privately held American company, Aloha Medicinals Inc. of Maui, Hawaii. This mixture is manufactured and distributed in the United States of America by Aloha Medicinals Inc under the Hawaiian Health Products brand name. The formula is composed of alcohol precipitated hot water extracts of *Lentinula edodes*, *Agaricus blazei*, *Grifola frondosa*, *Coriolus [Trametes] versicolor*, *Ganoderma lucidum* and two types of *Cordyceps sinensis* extracts, one from the mycelium and one from the culture broth of fermented *Cordyceps sinensis*. We tested this mixture concurrently with the accepted drug ***lamivudine*** in the cure of chronic hepatitis. With ***lamivudine*** therapy alone, the rate of hepatitis Be antigen converting from positive to negative is 10%-20% with a full years' course of therapy, and the antibody negativity increases by taking the medicine continuously year after year. Because of this long period of treatment, there is worry that the virus will induce genetic variation, as well as some patients discontinuing the therapy after time due to economic hardship. From February 2000 to August 2001, our research group used this mushroom polysaccharide tablet and ***lamivudine*** together to cure the hepatitis in 9 months. From April in 2001 to May in 2002, the research group used this mushroom polysaccharide tablet and ***lamivudine*** together to treat hepatitis and hyperlipidemia with good results. The Government Clinical Trial sanction number for this trial is 99-118. Each tablet is 0.5g total and contains 0.4g amylase reactable polysaccharides totally. The primary research results are as follows:

Key words: ***Immune-Assist***, mushroom polysaccharide, medicinal mushrooms, Hepatitis, Hyperlipidemia, HbsAL/HbeAg, ***Lamivudine***

Recipe and preparation method

1. Recipe: Lentinan, Maitake D-Fraction, Krestin, Catertabletar Fungus Polysaccharides, PSP, A. blazei Polysaccharide, binder, excipient, etc.
2. Preparation method: Prepare the formula and produce tablets according to reference [1], then package the tablets aseptically. Each tablet is 0.5g and has 0.4g polysaccharide in total.

Quality control standard

- 2.1 Character: The tablet is granular, light brown with a characteristic taste and smell.
- 2.2 Differentiation and verification:
 - (1) To the tablet 1.0g, add 2mol/L solution of hydrochloric acid, then dissolve both. After that, add ninhydrin about 2mg, heat up, so the solution changes from deep blue to light blue.
 - (2) To the tablet 1g, add water 20ml to dissolve. To 5ml of this solution, add silver nitrate 2.5ml and a black deposition of silver appears.
- 2.3 This product should measure up to all the medicine rules in the first addendum of "*The Codex in the People's Republic of China*" (Edition 1).
- 2.4 Shelf life experiment: Divide the tablets into 3 groups and store them under normal temperature. Then do character differentiation, check the solubility in water, the differentiation with equipment (GC, HPLC, etc) and the sterility in January, February, March, June, and December. All the datum accords with the related rules in the first addendum of "*The Codex in the People's Republic of China*" (Edition

1).

Toxicity experiment (presided over by Li Songhua MD at Daogen Medical College in Japan)

3.1 Acute toxicity experiment: 20 baby mice (20 ± 1 g each, half male half female), administer P.O. a solution of the polysaccharide tablet 3 times / 24 hours (0.5g for each mouse every time), total dose is 75g/kg/day. Maintain this dosage for 7 days and otherwise feed according to normal. At the end of the seven days all the mice are healthy and none show any signs of toxicity or abnormality. This short-term overdosage is approximately 835 times the normal adult dosage.

3.2 Long-term toxicity experiment: Choose 80 healthy adult mice, administer the solution P.O. at 10g/kg/day for 90 days. The mice have no abnormal characteristics and the tissues show no toxicity changes. This confirms that this product has little toxicity for long-term administration

Clinical observation

4.1 The group chose 60 patients, who have complete datum agreement for hepatitis since February 2000. These cases are all in accordance with the chronic hepatitis diagnostic standard which was made at the Beijing conference in 1995. That is to say, HbsAg and HbeAG are both positive. The patients are divided into two groups: the experimental group and the comparison group. The experimental group consists of 32 cases: 19 males and 13 females, whose age is from 16 y to 55 y and the average age is 32 y. Among these cases, the lightly sick number 10 individuals and the moderately sick are 22 in number. The comparison group consisted of 28 cases: 16 males and 12 females, with ages from 14 y to 53 y and an average age of 31 y. Among these cases, the lightly sick are 9 in number and the moderately sick number 19 individuals. As far as sex, age and degree of disease considerations, there is no apparent differentiation between the groups. ($P > 0.05$).

Treatment Methods

These two groups were all treated by Western medicine. **Lamivudine** was administered 100mg per day for a period of 9 months. Fufangyiganling or Ganlixinpian was also given 3 times a day, 4 tablets in each dose and continued for 6 months. The experimental group was treated the same, except for the addition of the mushroom polysaccharide tablets, 18 tablets per day divided into 3 doses, for a total daily dosage of 9 grams, and continuing for 9 months. During the treatment period, the two groups were checked for hepatic function every other month as well as checking the hepatic antigen and antibody system.

Treatment Standards Rating

4.2 Treatment efficacy standards:

- **Marked effect:** The main symptoms disappear, the swelling and inflammation of the liver and spleen subsides, hepatic function becomes normal, and one or more of either the HbsAL or HBeAg turns negative.
- **Moderate Effect:** The symptom improve, the swelling and inflammation of the liver and spleen subsides or becomes steady, the hepatic function approaches normal or becomes normal, but

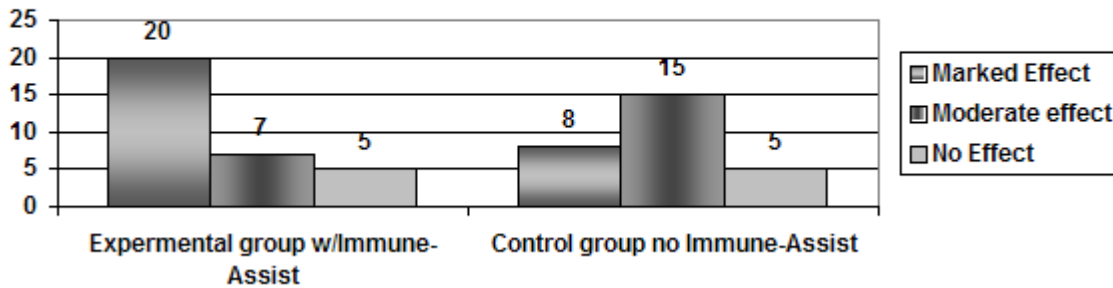
neither the HbsAL or the HBeAg turns negative.

- **Ineffective:** None of the above datum is approached.

Treatment result

4.3 In the experimental group, there are 20 cases of marked effect, 7 cases of Moderate Effect, and 5 cases considered ineffective, and the totally efficiency is 84.4%. In the comparison group, there are 8 cases of marked effect, 15 cases of Moderate Effect, 5 cases considered ineffective and the totally efficiency is 82.1%. The totally efficiency difference of these two groups has no apparent meaning (P>0.05). See Table 1 below:

Table 1 - Overall Effectivness



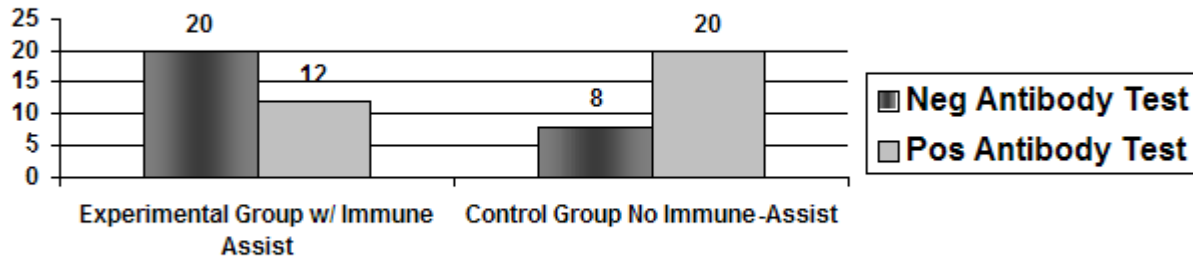
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Table 1 – Effectivness comparison of lamivudine alone compared to lamivudine & Immune-Assist

Comparison of rate of hepatitis antibodies turning from positive to negative

4.4 After 9 months of treatment, 20 cases of HBeAg in the experimental group turn negative (62.5%), while only 8 cases of HbeAg in comparison group turn negative (28.6%). The turning negative rate of these two groups has apparently meaning (P<0.05). See Table 2 below:

Table 2 - Antibody changing from Positive to Negative After 9 months of treatment



NOTE: The rate of change in the Antibody test from Positive to Negative is 62.5% for those patients treated with Immune-Assist compared to only 28.6% for those treated with Lamivudine alone.

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The patients whose HBeAg turns negative in these two groups continue to take Lamivudine for 3 months. There are 3 cases whose HBeAg returns to positive after stopping the medication in the experimental group, and 1 case in the comparison group. There is no apparent meaning.

Discussion

There are two treatment methods to cure hepatitis in the world today: one is to effect a cure through antiviral activity, the other is by assisting the immune system to fight the disease. When the inflammation is promoted by the reproduction of the virus, the main treatment method for chronic hepatitis is through the use of an antiviral drug. Up until now the efficiency of antiviral therapy is the decrease of HBV-DNA (<0.1pg/ml) and the elimination of HBeAg, since these results are related to changes of the inflammatory necrosis (or damage) and the reduction of communicability. After antiviral therapy, HBeAg and even HBsAg disappears. However, even though HBV-DNA still can be tested in serum and liver by PCR, this does mean that the disease still exists. Because of that, it has significant clinical meaning that we choose the turning negative method of HBeAg as the main guideline for curative effect.

The antiviral drugs, which are certified in international medical science, are *interferon* and *lamivudine*. But since *interferon* only can be used under the rigorous observation of veteran doctors, there are only a small portion of patients which can be treated in this manor out of the millions of cases worldwide. *Lamivudine* is similar to a nucleotide and causes a type of pyrimidine ramification. It is synthetically manufactured and is an L-structured artificial enantiomorph. It is a strong and safe inhibitor for the replication of HBV. It can play a great role in wide treatment usage, but it is not fit for all chronic hepatic infections. The medicine for all chronic infections should restrain the synthesis of HBV-DNA and eliminate CCCDNA, but does not rely on the immunological activity of the patients themselves. It has proven very difficult to produce that kind of medicine with both antiviral and immunomodulatory activity so far.

The HbsAL / HBeAg indicator, which we are researching, has one or more advantages. First, it can accurately choose medicines for treatment and let these medicines exert their advantages singly; and secondly, it can improve the curative effect concurrently with other medicines. However *lamivudine* takes a long time to play its disease resistance role singly (The turning negative rate of HBeAg is about 3-4 years to achieve 60% efficacy), and it is likely to induce virus genetic variation if taken for a long period of time (The variation of YMDD is easy to see). So we use the mixed medicines of the mushroom polysaccharides tablet and *lamivudine* to cure hepatitis in a shorter than usual time, and use Chinese medicine alone for a long-term follow-up. According to our experience (more than one and a half years), if we use the mushroom polysaccharide tablet and lamivudine together, the sensitivity to the *lamivudine* can be increased through the pretreatment with Chinese medicine. The multi-targeted antiviral activity of Chinese medicine (mushroom polysaccharide mixture) and the concurrent action of *lamivudine* can make up for the short comings of *lamivudine* alone, for example by allowing shorter periods of treatment, greater reduction in swelling and inflammation, and decreased toxic side effects.

According to the references cited below [2-4], edible fungi have many good biological functions. They can improve the patient's dormancy, increase the appetite, ameliorate fatigue, and regulate and enhance immunity. They can strengthen the body's nonspecific immunity, improve the secretory product of mucosa's secretor type IgA, increase the function of mononuclear-phagocyte and the activity of NK cell, maintain the immune balance, adjust the alcoholic damage to liver cell efficiency and accelerate

the restoration and regeneration of liver tissue cells. Besides these effects, edible fungi have great attenuation of virulence, improve the body's overall health condition, protect hemopoietic action of marrow, and lighten the damage of the esophagus. Edible fungi have certain curative effect for chemical liver damage and blood fat reduction, and can play an effective assistant function in the clinic. From this experiment, we find that the mushroom polysaccharide tablet can play an important roll in curing hepatitis, and this mushroom polysaccharide mixture (***Immune-Assist***) should be considered as an adjunct to conventional hepatitis treatment.

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